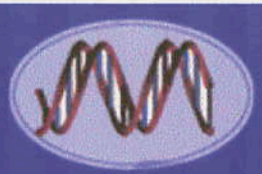
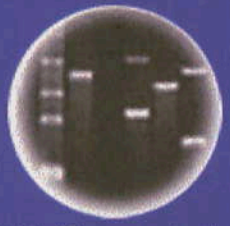


DNA
Genetic Code of Life

Entire Genetic Code
of a Bacteria



DNA Fingerprinting

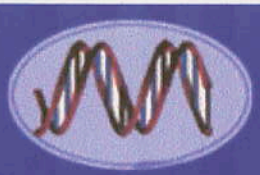
Cloning: Ethical Issues
and Future Consequences

Plants of Tomorrow

HC70A Winter 2008
Genetic Engineering in Medicine, Agriculture, and Law
Professor Bob Goldberg

Lecture 7
Twenty-First Century Genetic Engineering Applications

1 2.5 hr lecture on 2/21
+
1 2hr lecture on 2/28



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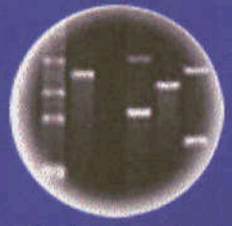


DNA Fingerprinting

Cloning: Ethical Issues
and Future Consequences

Plants of Tomorrow

THEMES

1. Review: Genetic Engineering Applications
2. Genetic Engineering of Bacteria
   - a. Drugs, Vaccines, & Antibiotics
   - b. Food Products (e.g., cheese)
   - c. Metabolism & Biofuel Production
   - d. Human Gene Delivery Systems
3. Genetic Engineering of Fungi
   - a. Drugs
   - b. Fermentation & Alcoholic Beverages

to pg 37

Stop 1 2.5hr lecture
Lots of discussion on Antibiotics

4. Genetic Engineering of Animals & Plants
   - a. Biopharming
   - b. Using Animals to Make Drugs
   - c. Using Plants to Make Vaccines
5. Genetic Engineering of Humans
   - a. Germ Cell vs. Somatic Cell Gene Therapy
   - b. In Vivo Gene Therapy
     - i. Cystic Fibrosis Gene Therapy
     - ii. Brain Cancer Gene Therapy
   - c. Ex Vivo Gene Therapy
     - i. ADA Gene Therapy
   - d. Cloning, Stem Cells, & Gene Therapy

Slides

Stop 2 hrs 2/28/08

APPLICATIONS OF GENETIC ENGINEERING

BASIC SCIENCES

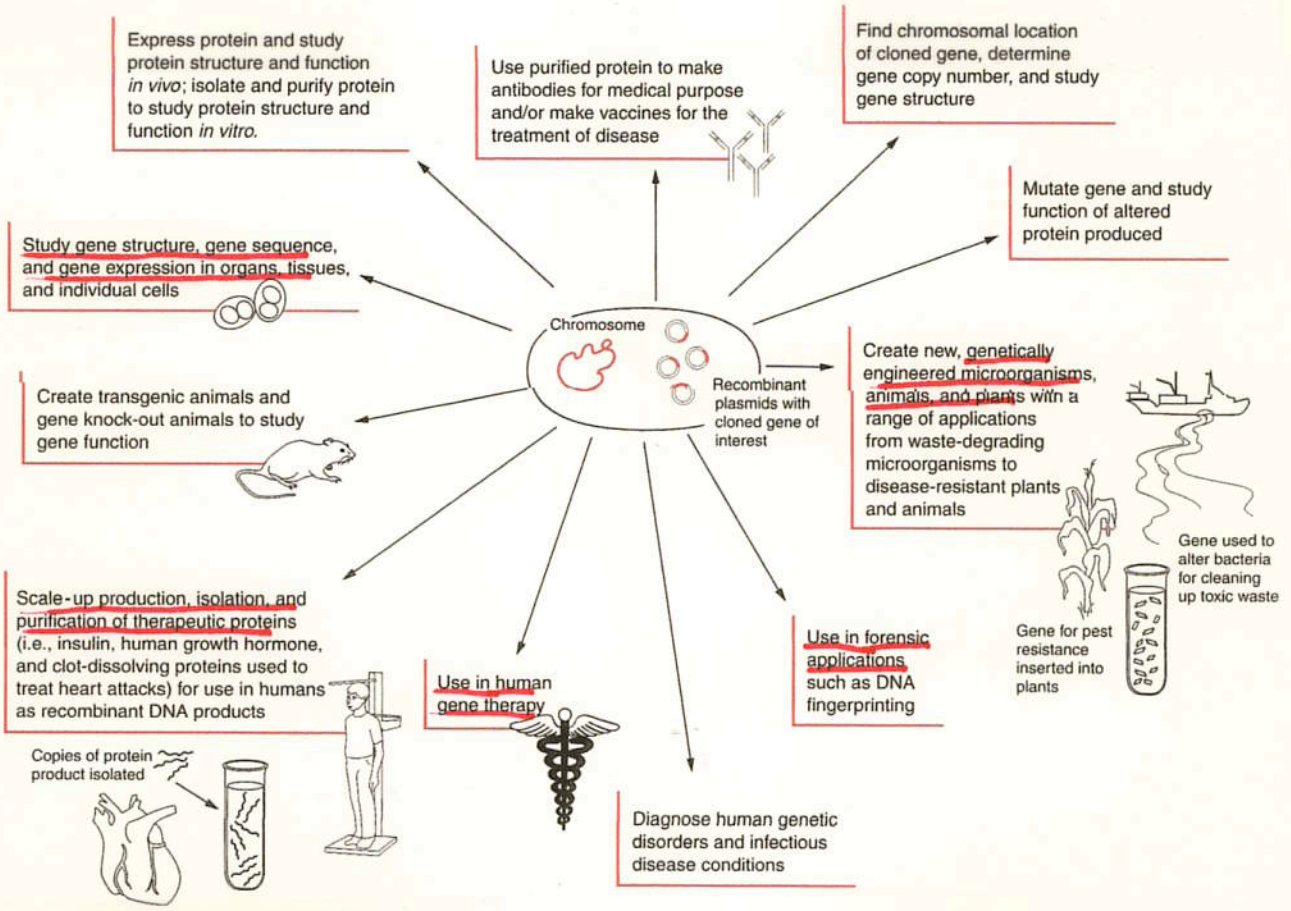


Figure 3.10 Applications of Recombinant DNA Technology

APPLIED USES

(2)

GENETIC ENGINEERING APPLICATIONS

Recombinant Nucleic Acids (DNA & RNA)

1. DNA Fingerprinting Probes/Templates – FORENSICS
2. DNA Probes/Templates – Genetic Disease Diagnosis, Paternity/Infectious Disease Pathogens
3. Gene Therapy
4. Anti-Sense/RNAi drugs
5. DNA computers!

RECOMBINANT VIRUSES

1. Gene Therapy Vehicles
2. Vaccines

Recombinant Microbes (Bacteria & Fungi/Molds)

1. Biofactories/Metabolic Engineering – Synthesis of Industrial Molecules
2. Drug Production – Human Proteins & Antibiotics
3. Enzymes/Protein Engineering – Food & Industrial Applications
4. Waste Remediation

Recombinant Animals

1. Disease Models (Mouse)
2. Drug Production (Whole Animals & Cells)
3. Improved FARM Animals For Food Production
4. TRANSgenic Fish for Fun/Pets & Food
5. TRANSgenic Mosquitos/Malaria Control

Recombinant Plants

1. Improved Crops/Higher Yields/Food production
2. Drug Production
3. Biofactories/Fuel
4. Bioremediation
5. Improved Foods/More Nutritious!

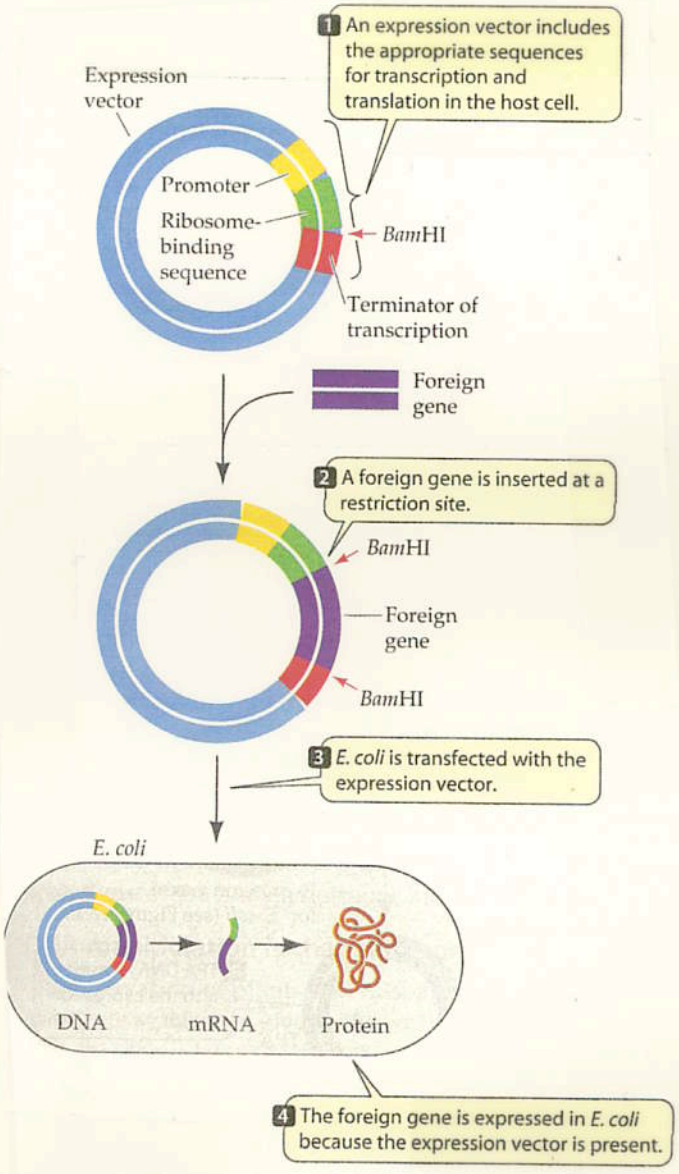
GENETIC ENGINEERING OF BACTERIA

GENETIC ENGINEERING BACTERIAL CELLS

Table 34.1 Bacteria

Major Group	Typical Examples		Key Characteristics
			ARCHAEBACTERIA
Archaebacteria	Methanogens, thermophiles, halophiles		Bacteria that are not members of the kingdom Eubacteria. Mostly anaerobic with unusual cell walls. Some produce methane. Others reduce sulfur.
			EUBACTERIA
Actinomycetes	*Streptomyces*, *Actinomyces*		Gram-positive bacteria. Form branching filaments and produce spores; often mistaken for fungi. Produce many commonly used antibiotics, including streptomycin and tetracycline. One of the most common types of soil bacteria; also common in dental plaque.
Chemoautotrophs	Sulfur bacteria, *Nitrobacter*, *Nitrosomonas*		Bacteria able to obtain their energy from inorganic chemicals. Most extract chemical energy from reduced gases such as H_2S (hydrogen sulfide), NH_3 (ammonia), and CH_4 (methane). Play a key role in the nitrogen cycle.
Cyanobacteria	*Anabaena*, *Nostoc*		A form of photosynthetic bacteria common in both marine and freshwater environments. Deeply pigmented; often responsible for "blooms" in polluted waters.
Enterobacteria	*Escherichia coli*, *Salmonella*, *Vibrio*		Gram-negative, rod-shaped bacteria. Do not form spores; usually aerobic heterotrophs; cause many important diseases, including bubonic plague and cholera.
Gliding and budding bacteria	Myxobacteria, *Chondromyces*		Gram-negative bacteria. Exhibit gliding motility by secreting slimy polysaccharides over which masses of cells glide; some groups form upright multicellular structures carrying spores called fruiting bodies.
Pseudomonads	*Pseudomonas*		Gram-negative heterotrophic rods with polar flagella. Very common form of soil bacteria; also contain many important plant pathogens.
Rickettsias and Chlamydias	*Rickettsia*, *Chlamydia*		Small, gram-negative intracellular parasites. *Rickettsia* life cycle involves both mammals and arthropods such as fleas and ticks; *Rickettsia* are responsible for many fatal human diseases, including typhus (*Rickettsia prowazekii*) and Rocky Mountain spotted fever. Chlamydial infections are one of the most common sexually transmitted diseases.
Spirochaetes	*Treponema*		Long, coil-shaped cells. Common in aquatic environments; a parasitic form is responsible for the disease syphilis.

EXPRESSION VECTORS ARE USED TO MAKE RECOMBINANT PROTEINS IN BACTERIAL CELLS



What switches? Terminators? Codon useage (for synthetic genes)?

RECOMBINANT PROTEINS MADE IN BACTERIA TO TREAT HUMAN DISEASES

Table 10.1 Some human proteins that have been produced by recombinant DNA technology for treating various disorders

Protein	Disorder(s)
α_1-Antitrypsin	Emphysema
Adrenocorticotropic hormone	Rheumatic diseases
B-cell growth factors	Immune disorders
Bactericidal/permeability-increasing protein	Infections
Brain-derived neurotrophic factor	Amyotrophic lateral sclerosis (Lou Gehrig's disease)
Calcitonin	Osteomalacia
Colony-stimulating factors	Cancer
Chorionic gonadatropin	Female infertility
Endorphins and enkephalins	Pain
Epidermal growth factor	Burns
Erythropoietin	Anemia, kidney disorders
Factor VIII	Hemophilia
Factor IX	Hemophilia
Growth hormone	Growth defects
Growth hormone-releasing factor	Growth defects
Hemoglobin	Anemia
Insulin	Diabetes
Insulin-like growth factor	Diabetes, renal failure
Interferons (α, β, γ)	Viral diseases, cancer, multiple sclerosis
Interleukins	Cancer, immune disorders
Interleukin-1 receptor	Asthma, rheumatoid arthritis
Lymphotoxin	Cancer
Macrophage-activating factor	Cancer
Nerve growth factor	Nerve damage
Platelet-derived growth factor	Atherosclerosis
Relaxin	Birthing
Serum albumin	Insufficient plasma proteins
Somatomedin C	Growth defects
Thyroid-stimulating hormone	Thyroid cancer
Tissue plasminogen activator	Blood clots
Tumor necrosis factor	Cancer
Urogastrone	Ulcers
Urokinase	Blood clots

IMPORTANCE OF ANTIBIOTICS & VACCINES IN COMBATING INFECTIOUS DISEASES

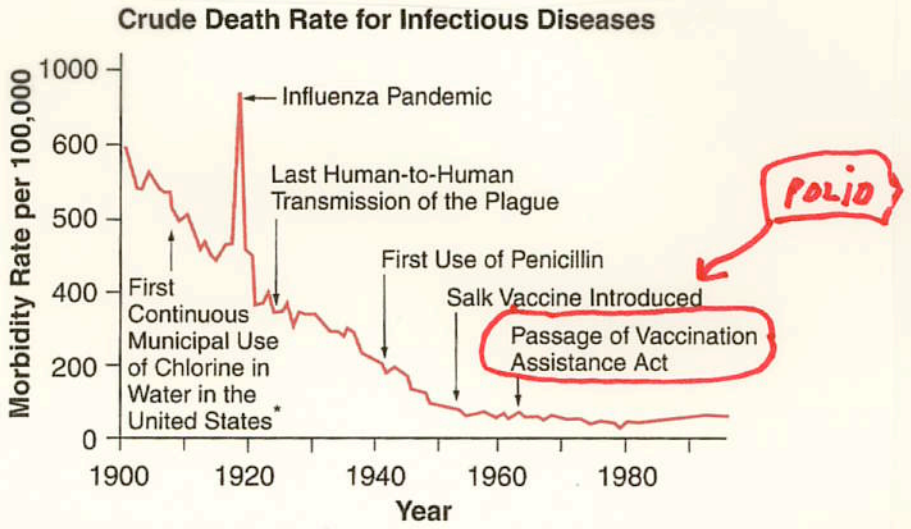


Figure 5.11 The Use of Antibiotics and Vaccines to Combat Infectious Diseases Caused by Microorganisms Even though the use of antibiotics and vaccines has decreased the incidence of human illness caused by microorganisms in the United States, new strains of microbes that show resistance to many popular antibiotics and vaccines are emerging. New antibiotics and vaccines are required to fight these microbes.

* The American Society for Microbiology Report: Congressional Briefing. Infectious Disease Threats, 2001.

RECOMINANT VACCINES CAN ALSO BE SYNTHESIZED

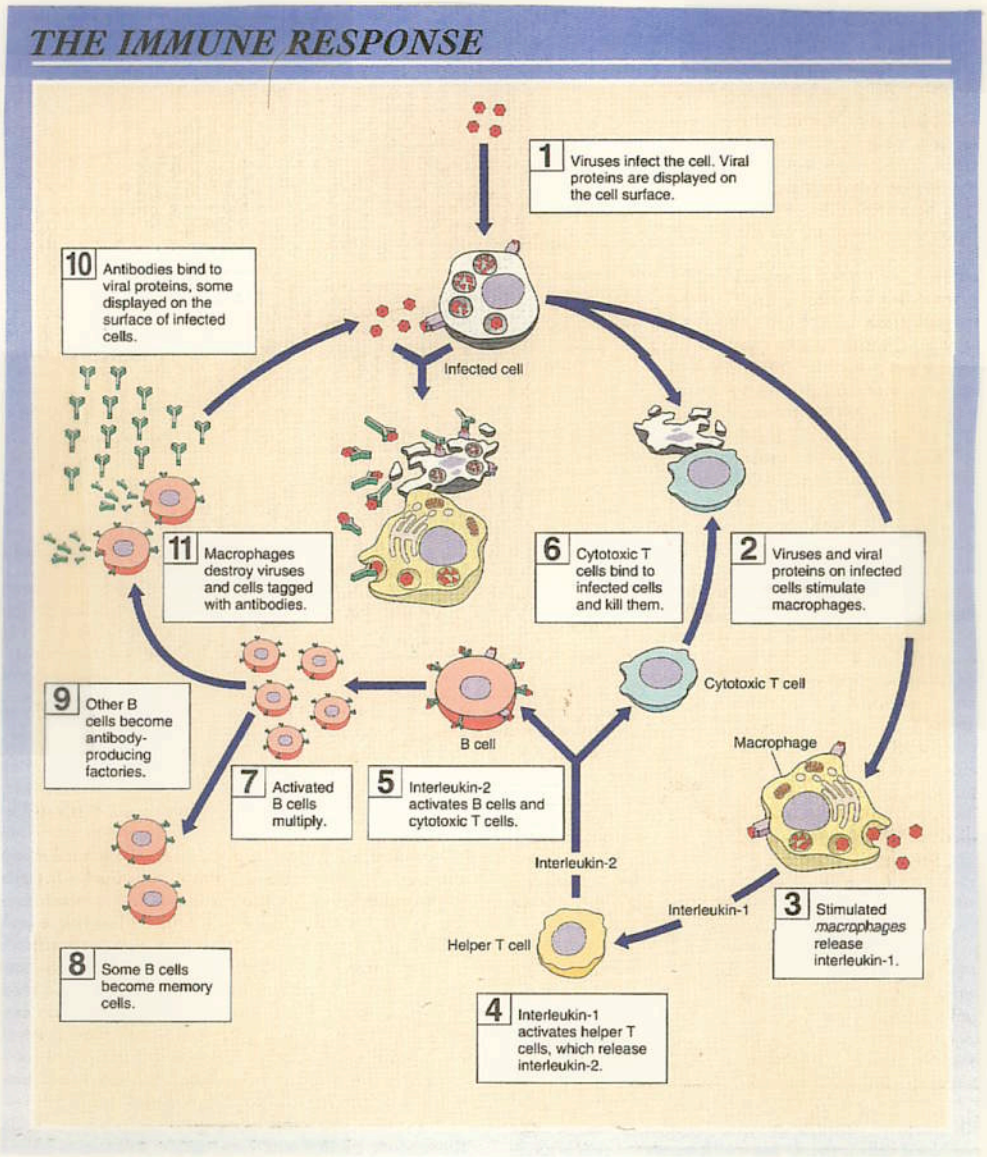


FIGURE 57.20
Overview of the specific immune response.

HUMAN BACTERIAL DISEASES

Table 34.2 Important Human Bacterial Diseases

Disease	Pathogen	Vector/Reservoir	Epidemiology
Anthrax	*Bacillus anthracis*	Animals, including processed skins	Bacterial infection that can be transmitted through contact or ingested. Rare except in sporadic outbreaks. May be fatal.
Botulism	*Clostridium botulinum*	Improperly prepared food	Contracted through ingestion or contact with wound. Produces acute toxic poison; can be fatal.
Chlamydia	*Chlamydia trachomatis*	Humans, STD	Urogenital infections with possible spread to eyes and respiratory tract. Occurs worldwide; increasingly common over past 20 years.
Cholera	*Vibrio cholerae*	Human feces, plankton	Causes severe diarrhea that can lead to death by dehydration; 50% peak mortality if the disease goes untreated. A major killer in times of crowding and poor sanitation; over 100,000 died in Rwanda in 1994 during a cholera outbreak.
Dental caries	*Streptococcus*	Humans	A dense collection of this bacteria on the surface of teeth leads to secretion of acids that destroy minerals in tooth enamel—sugar alone will not cause caries.
Diphtheria	*Corynebacterium diphtheriae*	Humans	Acute inflammation and lesions of mucous membranes. Spread through contact with infected individual. Vaccine available.
Gonorrhea	*Neisseria gonorrhoeae*	Humans only	STD, on the increase worldwide. Usually not fatal.
Hansen's disease (leprosy)	*Mycobacterium leprae*	Humans, feral armadillos	Chronic infection of the skin; worldwide incidence about 10–12 million, especially in Southeast Asia. Spread through contact with infected individuals.
Lyme disease	*Borrelia bergdorferi*	Ticks, deer, small rodents	Spread through bite of infected tick. Lesion followed by malaise, fever, fatigue, pain, stiff neck, and headache.
Peptic ulcers	*Helicobacter pylori*	Humans	Originally thought to be caused by stress or diet, most peptic ulcers now appear to be caused by this bacterium; good news for ulcer sufferers as it can be treated with antibiotics.
Plague	*Yersinia pestis*	Fleas of wild rodents: rats and squirrels	Killed ¼ of the population of Europe in the 14th century; endemic in wild rodent populations of the western U.S. today.
Pneumonia	*Streptococcus, Mycoplasma, Chlamydia*	Humans	Acute infection of the lungs, often fatal without treatment
Tuberculosis	*Mycobacterium tuberculosis*	Humans	An acute bacterial infection of the lungs, lymph, and meninges. Its incidence is on the rise, complicated by the development of new strains of the bacteria that are resistant to antibiotics.
Typhoid fever	*Salmonella typhi*	Humans	A systemic bacterial disease of worldwide incidence. Less than 500 cases a year are reported in the U.S. The disease is spread through contaminated water or foods (such as improperly washed fruits and vegetables). Vaccines are available for travelers.
Typhus	*Rickettsia typhi*	Lice, rat fleas, humans	Historically a major killer in times of crowding and poor sanitation; transmitted from human to human through the bite of infected lice and fleas. Typhus has a peak untreated mortality rate of 70%.

SEQUENCED VIRAL GENOMES PROVIDE TARGETS FOR VACCINES

TABLE 5.4 EXAMPLES OF MEDICALLY IMPORTANT VIRAL GENOMES THAT HAVE BEEN SEQUENCED RECENTLY

Virus	Human Disease or Illness	Year Sequenced
Ebola virus	Ebola hemorrhagic fever	1993
Hepatitis A virus	Hepatitis A	1987
Hepatitis B virus	Hepatitis B	1984
Hepatitis C virus	Hepatitis C	1990
Herpes symplex virus, Type I	Cold Sores	1988
Human immunodeficiency virus (HIV-1)	Acquired immunodeficiency syndrome (AIDS)	1985
Human papillomavirus	Cervical cancer	1985
Human poliovirus	Poliomyelitis	1981
Human rhinovirus	Common cold	1984
Variola virus	Smallpox	1992

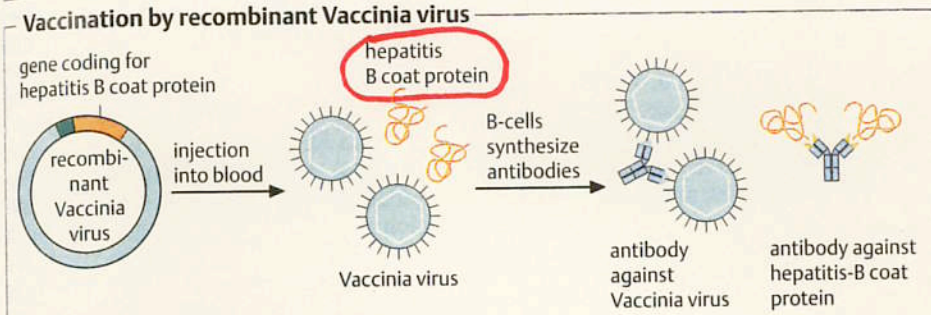
Adapted from: Haseltine, W. A. (2001): *Scientific American,* 285: 56–63.

USING GENETIC ENGINEERING TO MAKE VACCINES

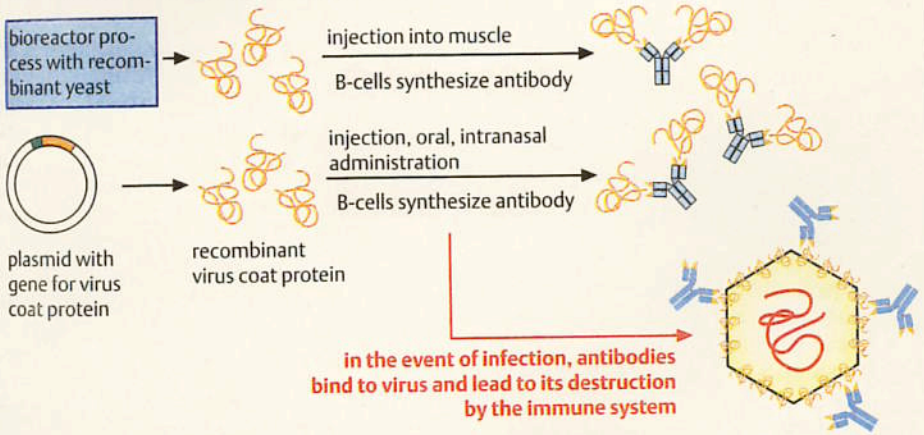
Recombinant vaccines (selection)

		antigen	status
viruses	hepatitis B	surface antigens	registered
	Herpes simplex type 2	surface antigens	clinical studies
	rabies vaccine	surface antigens	not registered
	yellow fever virus	surface antigens	preclinical studies
	AIDS virus	surface antigens	clinical studies
bacteria	*Streptococcus pneumoniae*	polysaccharide conjugate	registered
	Clostridium tetani	tetanus toxin	not registered
	Mycobacterium tuberculosis	surface antigens	clinical studies
parasites	*Plasmodium falciparum*	(malaria)	clinical studies
	Trypanosoma sp.	(sleeping sickness)	clinical studies
	Schistosoma mansoni	(bilharziosis)	clinical studies

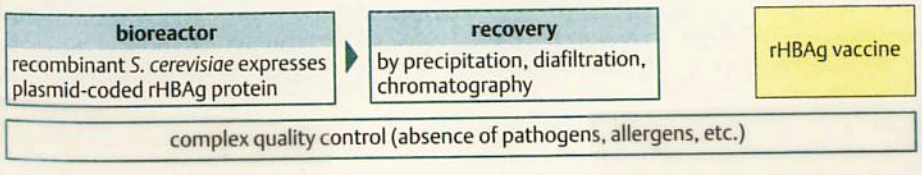
Vaccination by recombinant Vaccinia virus



Immunization with virus coat protein or DNA



Fermentation and recovery of recombinant hepatitis B vaccine



RECOMBINANT VACCINES ARE ALSO BEING DEVELOPED

Table 11.1 Human disease agents for which recombinant vaccines are currently being developed

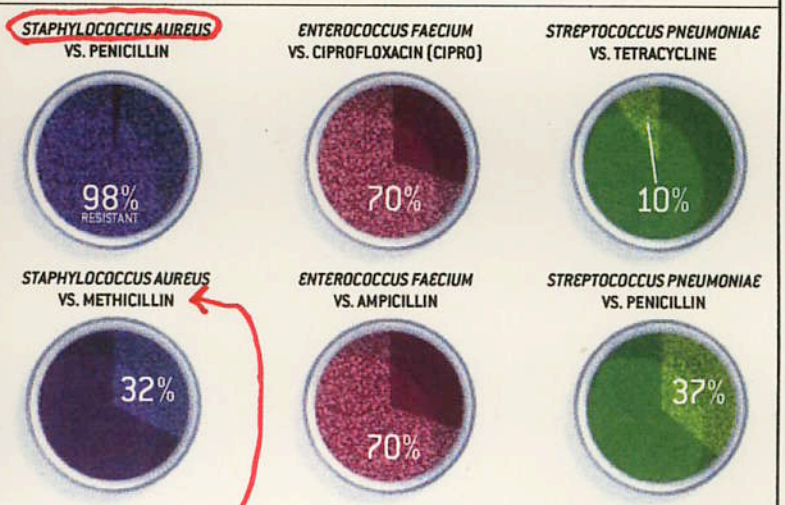
Pathogenic agent	Disease(s)
Viruses	
Varicella-zoster virus	Chicken pox
Cytomegalovirus	Infection in infants and immunocompromised patients
Dengue virus	Hemorrhagic fever
Hepatitis A virus	High fever, liver damage
Hepatitis B virus	Long-term liver damage
Herpes simplex virus type 2	Genital ulcers
Influenza A and B viruses	Acute respiratory disease
Japanese encephalitis virus	Encephalitis
Parainfluenza virus	Inflammation of the upper respiratory tract
Rabies virus	Encephalitis
Respiratory syncytial virus	Upper and lower respiratory tract lesions
Rotavirus	Acute infantile gastroenteritis
Yellow fever virus	Lesions of heart, kidney, and liver
Human immunodeficiency virus	AIDS
Bacteria	
Vibrio cholerae	Cholera
E. coli enterotoxin strains	Diarrheal disease
Neisseria gonorrhoeae	Gonorrhea
Haemophilus influenzae	Meningitis, septicemic conditions
Mycobacterium leprae	Leprosy
Neisseria meningitidis	Meningitis
Bordetella pertussis	Whooping cough
Shigella strains	Dysentery
Streptococcus group A	Scarlet fever, rheumatic fever, throat infection
Streptococcus group B	Sepsis, urogenital tract infection
Streptococcus pneumoniae	Pneumonia, meningitis
Clostridium tetani	Tetanus
Mycobacterium tuberculosis	Tuberculosis
Salmonella typhi	Typhoid fever
Parasites	
Onchocerca volvulus	River blindness
Leishmania spp.	Internal and external lesions
Plasmodium spp.	Malaria
Schistosoma mansoni	Schistosomiasis
Trypanosoma spp.	Sleeping sickness
Wuchereria bancrofti	Filariasis

CRITICAL TO FIGHT BIOWEAPONS!

Antibiotic Resistance is a major problem

RISING RESISTANCE

MANY ANTIBIOTICS are no longer effective against certain strains of bacteria, as these examples—collected from different hospitals in the late 1990s—show. One strain of *Staphylococcus aureus* found in Korea, for instance, is 98 percent resistant to penicillin (*top left*); another, found in the U.S., is 32 percent resistant to methicillin (*bottom left*). All these strains are not resistant to vancomycin, for now.



MRSA!

Antibiotics Synthesized in Microbes

Table 12.3 Some of the most common microbially synthesized antibiotics

Amikacin sulfate	Cefotaxime	Chlortetracycline	Kanamycin sulfate	Streptomycin sulfate
Amoxacillin	Cefoxitin	Clarithromycin	Lincomycin HCl	Teicoplanin
Ampicillin	Cefpodoxime proxetil	Clindamycin	Methicillin	Tetracycline HCl
Azithromycin	Ceftazidime	Erythromycin A	Oxytetracycline	Vancomycin HCl
Benzylpenicillin	Ceftriaxone	Flomoxef	Phenoxymethylpenicillin	
Cefaclor	Cefuroxime	Gentamicin sulfate	Rifampin	
Cefixime	Cephalexin	Imipenem	Spiramycin	

USING GENETIC ENGINEERING TO MAKE BETTER / MORE EFFECTIVE ANTIBIOTICS

Occurrence

taxonomic group	relative number (%)
Actinomycetes	50
other bacteria	10
fungi	20
lichens	1
algae	2
plants	15
animals	2

~25000 compounds from nature

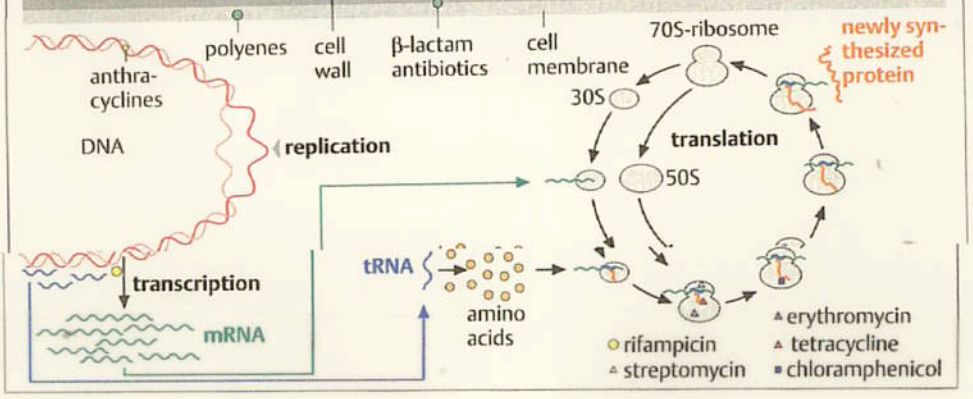
Systemic antibiotics (2001)

type	value (billion US $)
cephalosporins	6.7
penicillins	4.6
chinolones (synthetic)	4.6
macrolides	4.3
tetracyclines	0.7
aminoglycosides	0.6
peptide antibiotics, glycopeptides	0.5
other	2.2
total	24.2

Classification by chemical structure

1	**carbohydrate antibiotics**	aminoglycosides	streptomycin (medicine), kasugamycin (rice fungicide)
2	**macrocyclic lactones**	macrolides polyene antibiotics ansamycines	erythromycin (medicine) pimaricin (cheese production) rifamycin (against tuberculosis)
3	**chinones and related antibiotics**	tetracyclines anthracyclines	tetracycline, chlorotetrycycline (medicine, feed antibiotic) doxorubicin (cancer therapy)
4	**amino acid and peptide antibiotics**	amino acid derivatives β-lactam antibiotics peptide antibiotics chromopeptides glycopeptides	cyclosporin (organ transplantation) phosphinothricin (plant protection) penicillins, cephalosporins (medicine) bacitracin (medicine), virginiamycin (feed antibiotic) actinomycin (cancer therapy), bleomycin (cancer therapy), vancomycin (medicine), avoparcine (cattle feed antibiotic)
5	**N-heterocyclic compounds**	nucleoside antibiotics	polyoxins, blasticidin S (fungicides for plant protection)
6	**O-heterocyclic compounds**	polyether antibiotics	monensin (chicken feed)
7	**alicyclic compounds**	cycloalkane derivatives	cycloheximide (leaf fungicide)
8	**aromatic antibiotics**	benzene derivatives	chloramphenicol (medicine) griseofulvin (fungicide)

Antibiotics – point of attack



USING DNA SEQUENCES TO IDENTIFY BACTERIAL & VIRAL PATHOGENS

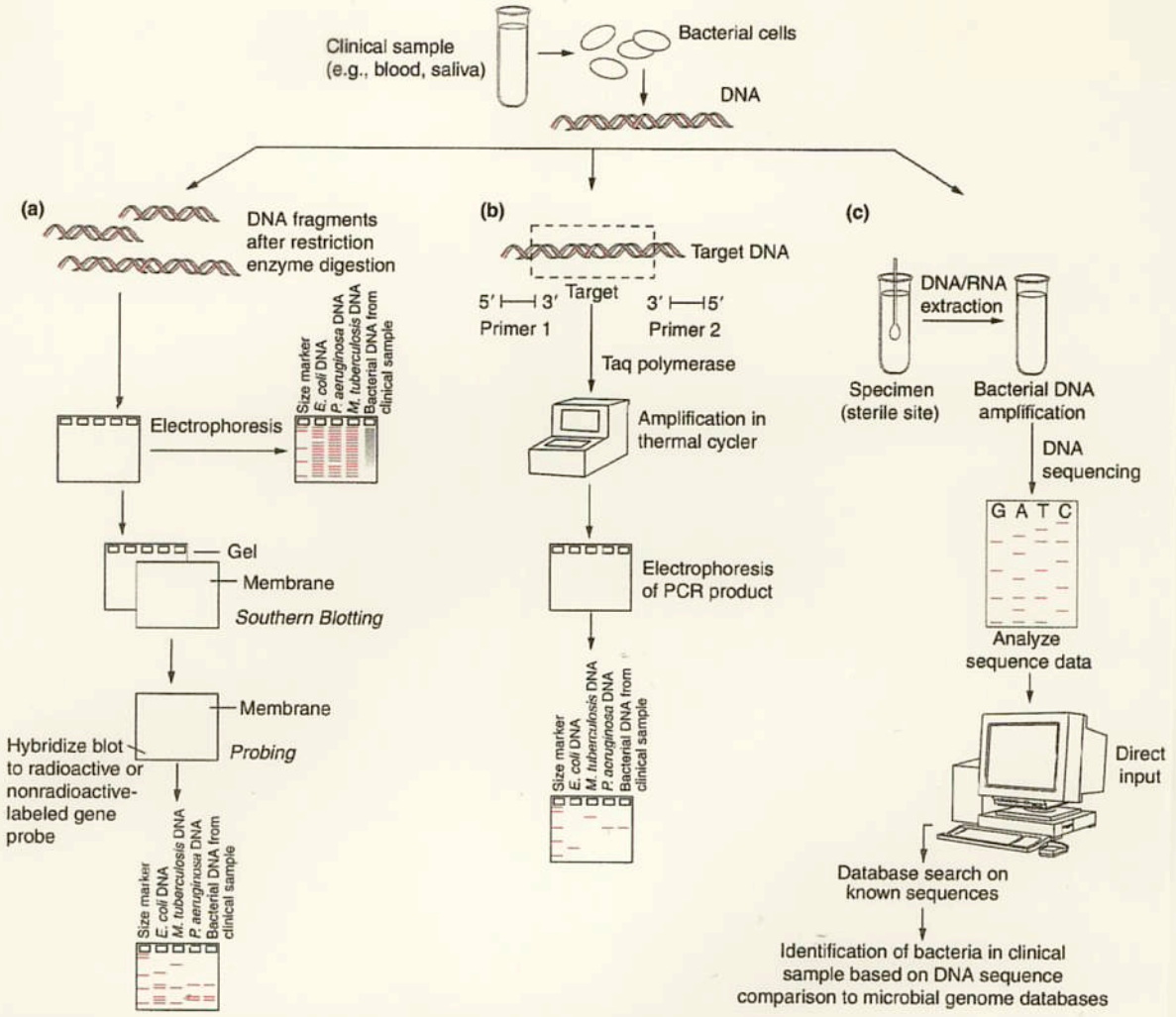


Figure 5.16 Using Molecular Techniques to Identify Bacteria Many molecular techniques are available for identifying bacteria. (a) RFLP is one such technique. For some pathogens, isolated DNA (which may come from a clinical sample such as blood or saliva) can be subjected to restriction enzyme digestion and separation by agarose gel electrophoresis. Banding patterns of DNA fragments can be compared to reference strains of known bacteria to allow for a positive identification. In this example, bacterial DNA isolated from the clinical sample matches *P. aeruginosa* (b) PCR can also be used for bacterial identification. PCR has the advantage of being much more sensitive than RFLP analysis; therefore, only small amounts of clinical samples and small amounts of DNA are required. The sensitivity of PCR also makes it possible to identify small amounts of DNA from just a few cells allowing for early treatment of an infection. (c) DNA sequencing strategies are also commonly used for microbial identification.

e.g. Food Poisoning Bacteria
BioWeapon / Anthrax

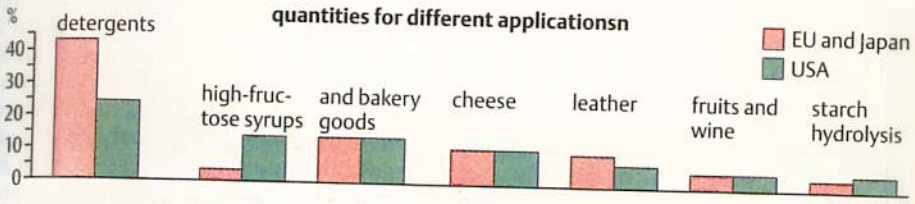
GENOMICS (META) + GENE DISCOVERY

NEW BACTERIA ANTIBIOTICS / METAGENOMICS

BACTERIA & OTHER MICROBES ARE THE SOURCE OF MANY DIFFERENT PRODUCTS

Enzymes as additives in industry

application	enzyme type	organisms (examples)	market size (% of total)	economic advantage
detergents	proteases, cellulases, lipases	*Bacillus licheniformis* *Aspergillus nidulans* *Trichoderma reesei*	40	1
starch hydrolysis	α-amylase	*Bacillus amyloliquefaciens*	5	3, 4
glucose isomerization	glucose isomerase	*Streptomyces venezuelae*	7	1, 3
beer brewing	amylase	*Bacillus subtilis*	3	3, 4
fruit processing, wine	cellulases, hemicellulases, pectinases	*Aspergillus niger*	5	3, 4, 5, 6
flour, bakery goods	α-amylase, proteases	*Aspergillus oryzae*	8	1, 3
cheese manufacture, aroma	proteases, chymosin, lipases	animal rennin, *Rhizomucor miehei*, *Saccharomyces cerevisiae*	12	2
silage and animal feed	phytases	*Aspergillus niger*	8	3
paper and textiles	α-amylase, lipase	*Bacillus, Humicola*	2	4
leather treatment	proteases	*Aspergillus oryzae*	10	1, 7



quantities for different applicationsn

process/application	enzyme cost per unit quantity (US $)
starch liquefaction	ca. $ 2 per t starch
glucose from starch	$ 3.5 per t starch
isomerization of glucose	$ 6 per t starch
HFS in USA	$ 6–7 per t starch
ethanol	$ 1 per t starch
beer	$ 0.1 per 100 L
bakery goods USA	$ 0.1 per 100 kg flour
bakery goods EU	$ 0.1–0.5 per 100 kg flour
fruit juice	$ 0.1–0.5 per 100 L juice
wine	$ 0.1–0.5 per 100 L wine
stabilization of fruit lemonade by glucose oxidase	$ 0.3–0.8 per 1000 L
cheese manufacture	$ 0.05 per 100 L milk
detergents	$ 0.05 per kg detergent
leather tanning	$ 1.2–3 per t skin

important goals in application technology

1 higher product quality
2 improved taste
3 better yields
4 reduced process costs
5 better filtration
6 better conservation
7 improved working conditions, reduced environmental load

IMPROVED and/or MANIPULATED BY RECOMBINANT DNA!

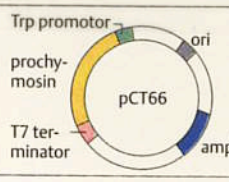
RECOMBINANT CHYMOSIN IS USED TO MAKE CHEESE

Composition of milk

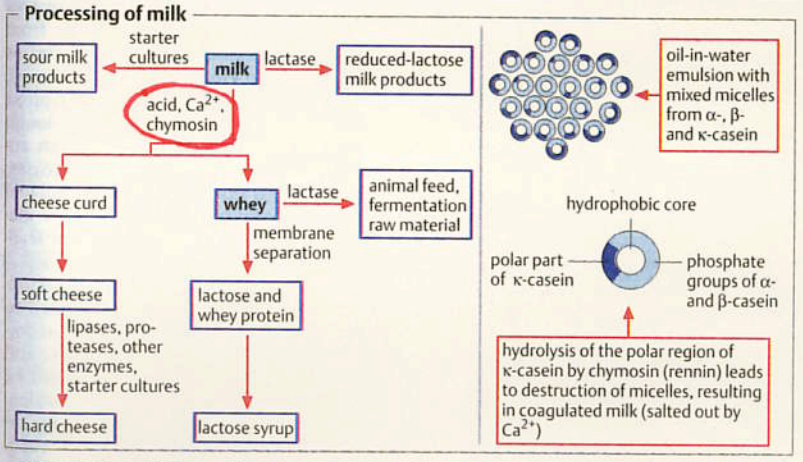
	milk (%)	whey (%)
water	~ 88	~ 94
fat	~ 3–4	~ 0.5
protein	~ 3.3	~ 1
casein	~ 2.6	–
lactose	–	~ 4.8

Plasmid

for the expression of chymosin in *E. coli*



Processing of milk



Manufacture of chymosin

native	microbial	recombinant
stomachs of young animals	**preculture**	**recombinant microorganism**
cutting, activation at pH < 5	high-yield mutants of *Mucor miehei* or *M. pusillus*	*Escherichia coli*
extraction	**bioreactor**	**bioreactor**
salt water, 14 d	dextrose syrup, soy meal, 30 °C, 72 h	maltodextrins, 37 °C, 36 h
purification	**purification**	**purification**
ultrafiltration standardization	separation of mycelium, re-verse osmosis, precipitation	isolation of inclusion bodies, Tritron-X100/EDTA, urea-/alkali-extract, ion-exchange chromato-graphy, acid treatment
200 U/kg stomach	5000 U/m³ in 72 h	20000 U/m³ in 36 h

Chymosin Acts on Milk Protein to coagulate Milk → Cheese!

Is Cheese A GMO?

Chymosin in Cheesemaking

(1) ~ 80-90% of cheeses are made with Recombinant Chymosin

(2) Approved for use in Cheesemaking by FDA – 1992

(3) Not different from non-recombinant Chymosin – ∴ GRAS – Generally Regarded as Safe & not labeling needed – because not an additive & not different from non-recombinant chymosin!

Is Cheese Made using a GMO?

Industry ads claim that Recombinant Chymosin is "Kosher" & "Vegetarian"

PROTEIN ENGINEERING - EVOLUTION IN A TEST TUBE!!

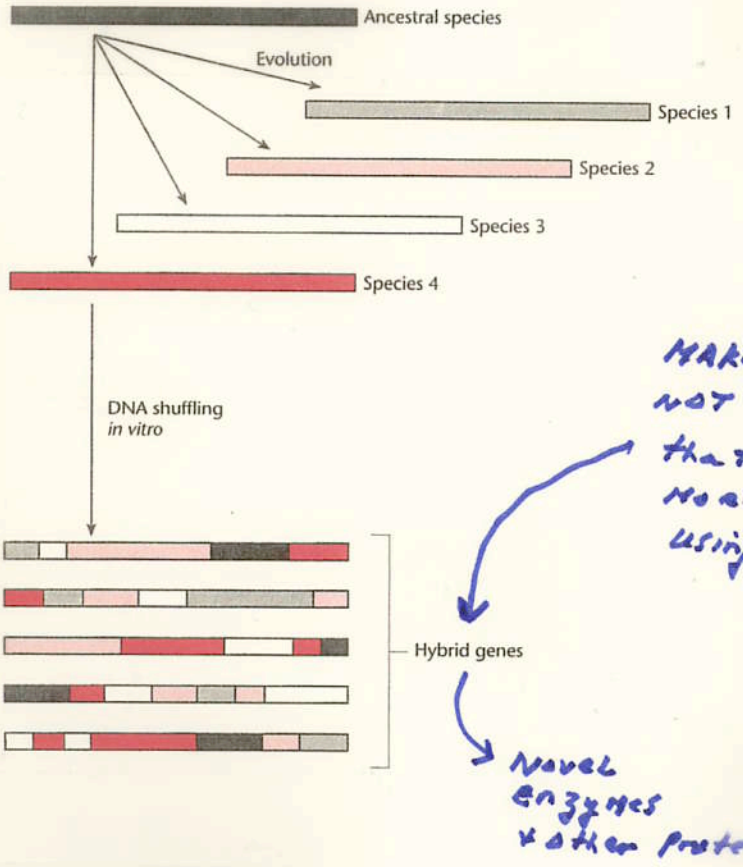


Fig. 14.14 Schematic representation of gene shuffling.

e.g.) more effective chymosin!

Useful Bacterial Metabolites that can be Engineered

Table 5.1 Examples of Primary and Secondary Metabolites Produced by Fermentation

Primary Metabolites	Secondary Metabolites
Amino acids	Antibiotics
Vitamins	Pigments
Nucleotides	Toxins
Polysaccharides	Alkaloids
Ethanol Acetone Butanol Lactic acid	Many active pharmacological compounds (e.g., the immunosuppressor cyclosporin, hypotensive compound dopastin)

Organic Chemical	Microbial Sources	Selected Uses
Acetic acid	*Acetobacter*	Industrial solvent and intermediate for many organic chemicals, food acidulant
Acetone	*Clostridium*	Industrial solvent and intermediate for many organic chemicals
Acrylic acid	*Bacillus*	Industrial intermediate for plastics
Butanol	*Clostridium*	Industrial solvent and intermediate for many organic chemicals
2,3-Butanediol	*Aerobacter, Bacillus*	Intermediate for synthetic rubber manufacture, plastics and antifreeze
Ethanol	*Saccharomyces*	Industrial solvent, intermediate for vinegar, esters and ethers, beverages
Formic acid	*Aspergillus*	Textile dyeing, leather treatment, electroplating, rubber manufacture
Fumaric acid	*Rhizopus*	Intermediate for synthetic resins, dyeing, acidulant, antioxidant
Glycerol	*Saccharomyces*	Solvent, plasticizer, sweetener, explosives manufacture, printing, cosmetics, soaps, antifreeze
Glycolic acid	*Aspergillus*	Textile processing, pH control, adhesives, cleaners
Isopropanol	*Clostridium*	Industrial solvent, cosmetic preparations, antifreeze, inks
Lactic acid	*Lactobacillus, Streptococcus*	Food acidulant, dyeing, intermediate for lactates, leather treatment
Methylethyl ketone	*Chlamydomonas*	Industrial solvent, intermediate for explosives and synthetic resins
Oxalic acid	*Aspergillus*	Printing and dyeing, bleaching agent, cleaner, reducing agent
Propylene glycol	*Bacillus*	Antifreeze, solvent, synthetic resin manufacture, mold inhibitor
Succinic acid	*Rhizopus*	Manufacture of lacquers, dyes and esters for perfumes

Optimize using genetic Engineering

BACTERIAL METABOLIC PATHWAYS CAN BE ENGINEERED TO OPTIMIZE PRODUCTION OF NOVEL INDUSTRIAL PRODUCTS

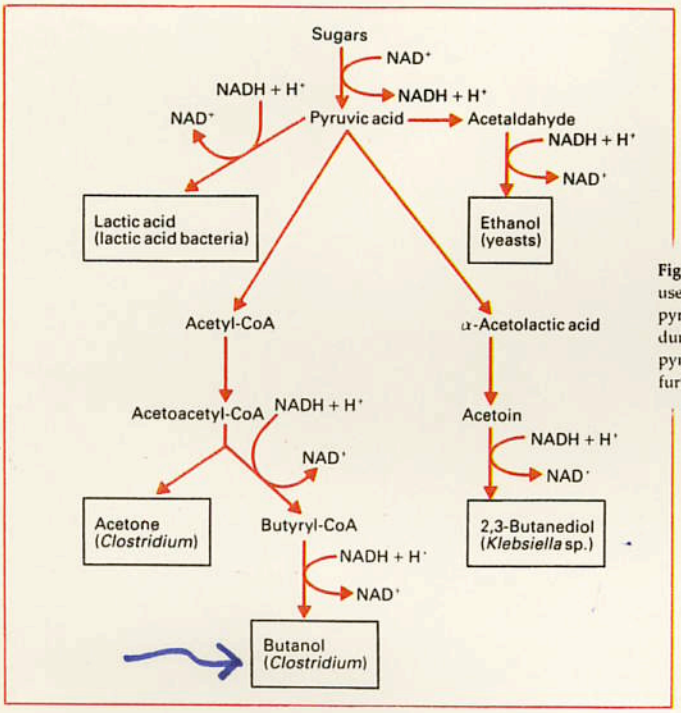


Fig. 6.5 The formation of commercially useful metabolic end-products. Note that pyridine nucleotide cofactors are reduced during the conversion of sugars to pyruvate and subsequently oxidized by further metabolism of pyruvate.

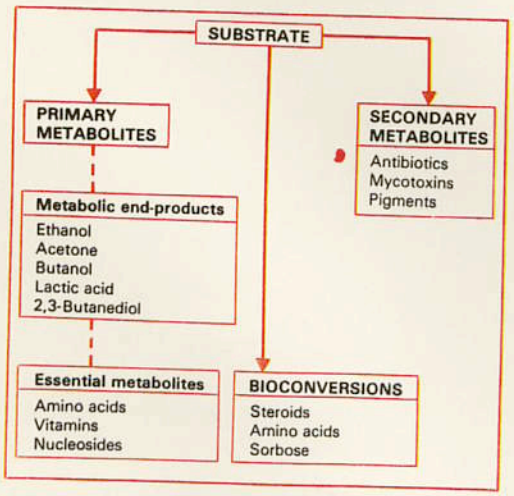


Fig. 6.4 The different classes of low-molecular-weight compounds synthesized by microorganisms.

These pathways can be optimized &/or changed by adding genes on plasmids that encode novel enzymes

e.g. Maxygen® → more diverse
- Protein Engineering
gene shuffling
protein evolution

ENGINEERING E.coli to make Biofuel

nature Vol 451|3 January 2008|doi:10.1038/nature06450

LETTERS

Non-fermentative pathways for synthesis of branched-chain higher alcohols as biofuels

Shota Atsumi[1], Taizo Hanai[1] & James C. Liao[1,2]

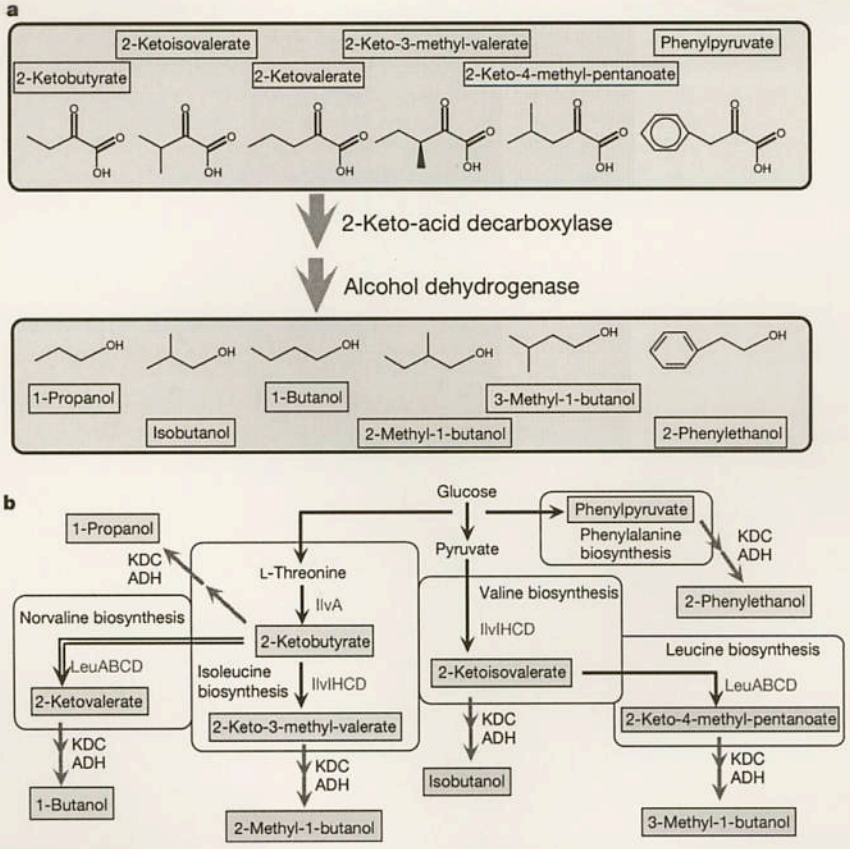
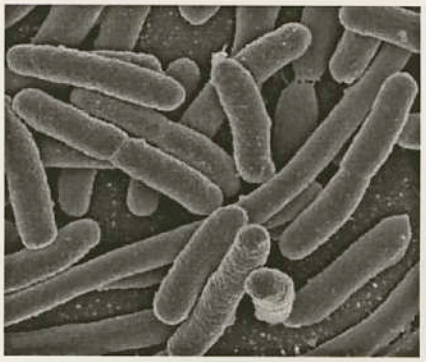


Figure 1 | Production of higher alcohols through the synthetic non-fermentative pathways. a, Various 2-keto acid precursors lead to corresponding alcohols through 2-ketoacid decarboxylase and alcohol dehydrogenase. **b,** The synthetic networks for the non-fermentative alcohol production in engineered *E. coli*. Red arrows represent the 2-keto acid decarboxylation and reduction pathway. Blue enzyme names represent amino acid biosynthesis pathways. The double lines represent a side pathway leading to norvaline and 1-butanol biosynthesis.

Efficient Biofuel Made From Genetically Modified E. Coli Bacteria

ScienceDaily (Jan. 7, 2008) — Researchers at the UCLA Henry Samueli School of Engineering and Applied Science have developed a new method for producing next-generation biofuels by genetically modifying Escherichia coli bacteria to be an efficient biofuel synthesizer. The method could lead to mass production of these biofuels.



Researchers have genetically modified Escherichia coli bacteria to make it an efficient biofuel synthesizer. (Credit: Rocky Mountain Laboratories, NIAID, NIH)

See also:

Plants & Animals
- Bacteria
- Agriculture and Food

Matter & Energy
- Petroleum
- Alternative Fuels

Earth & Climate
- Energy and the Environment
- Renewable Energy

Reference
- Common ethanol fuel mixtures
- Alternative fuel vehicle
- Biomass
- Ethanol fuel

Concerns about long-term fossil fuel availability, coupled with environmental problems resulting from their production and use, have spurred increased efforts to synthesize biofuels from renewable resources.

Biofuels, like commercially available ethanol, are produced from agricultural products such as corn, sugarcane or waste cellulose. Ethanol, however, has limitations — it is not as efficient as gasoline and must be mixed with gas for use as a transportation fuel. It also tends to absorb water from its surroundings, making it corrosive and preventing it from being stored or distributed in existing infrastructure without modification.

Higher-chain alcohols have energy densities close to gasoline, are not as volatile or corrosive as ethanol, and do not readily absorb water. Furthermore, branched-chain alcohols, such as isobutanol, have higher-octane numbers, resulting in less knocking in engines. Isobutanol or C5 alcohols have never been produced from a renewable source with yields high enough to make them viable as a gasoline substitute.

A new strategy has been developed by UCLA professor of chemical and biomolecular engineering James Liao, postdoctoral fellow Shota Atsumi and visiting professor Taizo Hanai.

"These alcohols are typically trace byproducts in fermentation," Liao said. "To modify an organism to produce these compounds usually results in toxicity in the cell. We bypassed this difficulty by leveraging the native metabolic networks in E. coli but altered its intracellular chemistry using genetic engineering to produce these alcohols."

The research team modified key pathways in E. coli to produce several higher-chain alcohols from glucose, a renewable carbon source, including isobutanol, 1-butanol, 2-methyl-1-butanol, 3-methyl-1-butanol and 2-phenylethanol.

This strategy leverages the E. coli host's highly active amino acid biosynthetic pathway by shifting part of it to alcohol production. In particular, the research team achieved high-yield, high-specificity production of isobutanol from glucose.

This new strategy opens an unexplored frontier for biofuels production, both in coli and in other microorganisms.

"The ability to make these branched-chain higher alcohols so efficiently is surprising," Liao said. "Unlike ethanol, organisms are not used to producing these unusual alcohols, and there is no advantage for them to do so. The fact that they can be made by E. coli is even more surprising, since E. coli is not a promising host to tolerate alcohols. These results mean that these unusual alcohols in fact can be manufactured as efficiently as what evolved in nature for ethanol. Therefore, we now can explore these unusual alcohols as biofuels and are not bound by what nature has given us."

UCLA has licensed the technology through an exclusive royalty-bearing license to Gevo Inc., a Pasadena, Calif.-based company founded in 2005 and dedicated to producing biofuels.

"Given that part of UCLA's mission is to transfer technologies to the commercial sector to benefit the public, we are excited at the prospect that this UCLA-developed technology may play a key role in addressing climate change and energy independence," said Earl Weinstein, assistant director of the UCLA Office of Intellectual Property. "It has been a pleasure to work with the team at Gevo on this deal, and we look forward to an ongoing relationship with them".

"This discovery leads to new opportunities for advanced biofuel development," said Patrick Gruber, Gevo's chief executive officer. "As the exclusive licensee of this technology, we can further our national interests in developing advanced renewable resource-based fuels that will help address the issues of climate change and future energy needs while creating a significant competitive advantage."

Liao has joined Gevo's scientific advisory board. In this role, he will continue to provide technical oversight and guidance during the commercial development of this technology.

"Dr. Liao's input will be invaluable as we scale up the commercial applications made possible by this breakthrough in technology and bring advanced biofuels to market," said Matthew Peters, chief scientific officer of Gevo.

Full details of the research appear in the Jan. 3 issue of the journal Nature.

The research was supported in part by the UCLA–Department of Energy Institute for Genomics and Proteomics and the UCLA–NASA Institute for Cell Mimetic Space Exploration.

Adapted from materials provided by University of California, Los Angeles.

ENGINEERING E. coli to synthesize INDIGO – The Major Blue Dye for Jeans & other clothes & uses

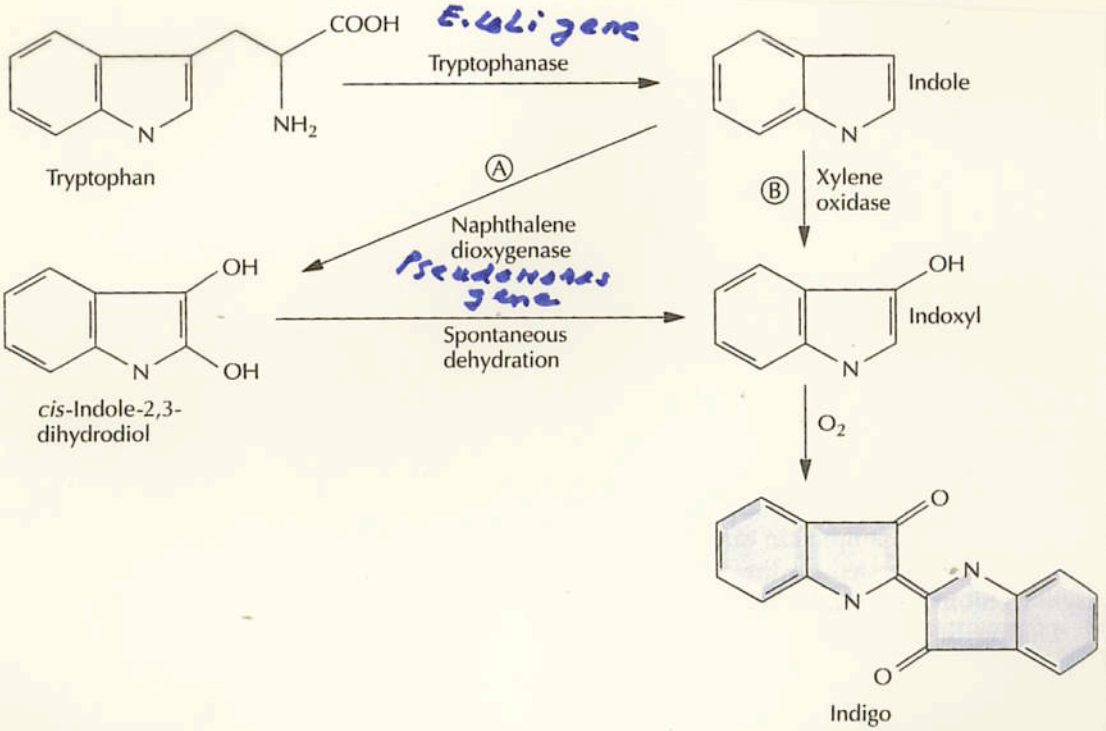


Figure 12.8 Indigo biosynthesis from tryptophan in genetically engineered *E. coli*. Tryptophanase is an *E. coli* enzyme. In pathway A, the naphthalene dioxygenase is derived from the NAH plasmid; in pathway B, the xylene oxidase is from the TOL plasmid. *E. coli* transformants that synthesize indigo contain either pathway A or B but not both pathways.

$200 M/year industry

Indigo previously obtained from plants!

POLLUTANTS IN ENVIRONMENT

TABLE 9.1 TWENTY OF THE MOST COMMON CHEMICAL POLLUTANTS IN THE ENVIRONMENT

Chemical Pollutant	Source
Benzene	Petroleum products used to make plastics, nylon, resins, rubber, detergents, and many other materials
Creosote	Wood preservative to prevent rotting
Cyanide	Mining processes and manufacturing of plastics and metals
Dioxin	Pulp and paper bleaching, waste incineration, and chemical manufacturing processes
Methyl t-butyl ether (MTBE)	Fuel additive, automobile exhaust, boat engines, leaking gasoline tanks
Naphthalene	Product of crude oil and petroleum
Nitriles	Rubber compounds, plastics, and oils
Perchloroethylene	Dry cleaning agent
Pesticides (atrazine, carbamates, chlordane, DDT) and herbicides	Chemicals used to kill insects (pesticides) and weeds (herbicides)
Phenol and related compounds (chlorophenols)	Wood preservatives, paints, glues, textiles
Polychlorinated biphenyls (PCBs)	Electrical transistors, cooling and insulating systems
Polycyclic aromatic hydrocarbons (PAHs) and polychlorinated hydrocarbons	Incineration of wastes, automobile exhaust, oil refineries, and leaking oil from cars
Polyvinylchloride	Plastic manufacturing
Radioactive compounds	Research and medical institutions and nuclear power plants
Surfactants (detergents)	Manufacturing of paints, textiles, concrete, paper
Synthetic estrogens (ethinyl estradiol)	Female hormone (estrogen)-related compounds created by a variety of industrial manufacturing processes
Tetrachloroethylene and trichloroethylene	Dry cleaning chemicals and degreasing agents
Toluene	Petroleum component present in adhesive, inks, paints, cleaners, and glues
Trace metals (arsenic, cadmium, chromium, copper, lead, mercury, silver)	Car batteries and metal manufacturing processes
Trinitrotoluene (TNT)	Explosive used in building and construction industries

BIOREMEDIATION

BACTERIA CAN BE ENGINEERED TO HAVE NOVEL DEGRADATIVE PATHWAYS FOR BIOREMEDIATION

PLASMIDS

Table 13.1 *Pseudomonas* plasmids, their degradative pathways, and their sizes

Name of plasmid	Compound(s) degraded	Plasmid size (kb)
SAL	Salicylate	60
SAL	Salicylate	72
SAL	Salicylate	83
TOL	Xylene and toluene	113
pJP1	2,4-D	87
pJP2	2,4-D herbicide	54
pJP3	2,4-D	78
CAM	Camphor	225
XYL	Xylene	15
pAC31	3,5-Dichlorobenzoate	108
pAC25	3-Chlorobenzoate	102
pWWO	Xylene and toluene	176
NAH	Naphthalene	69
XYL-K	Xylene and Toluene	135

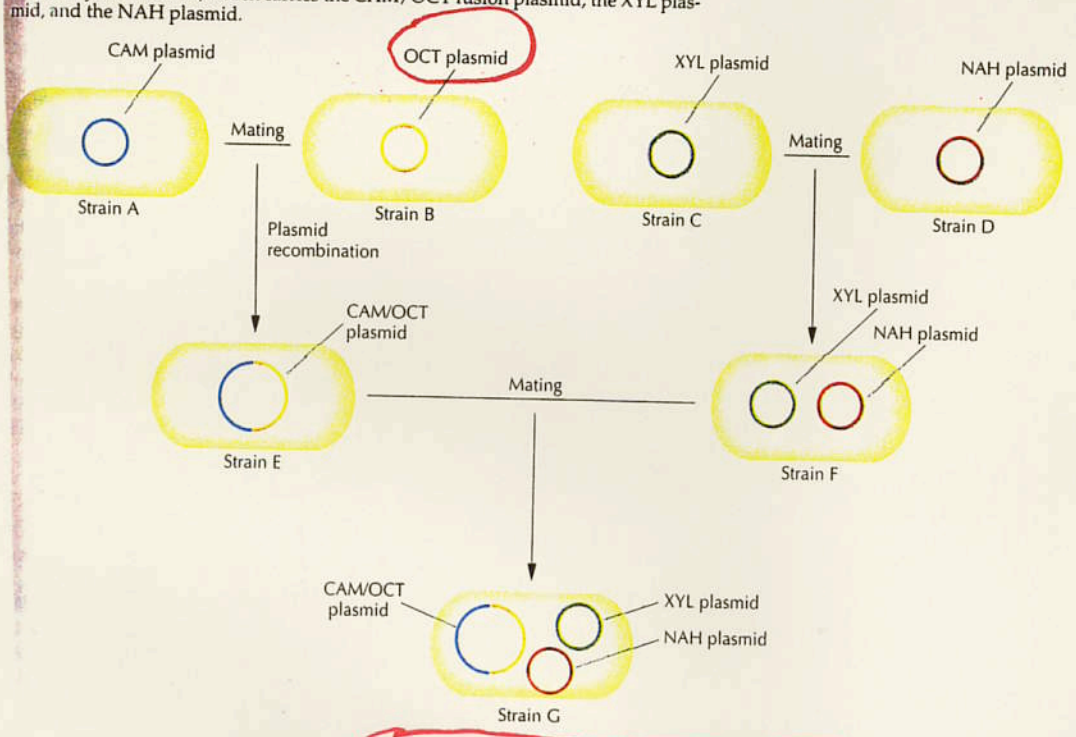
Adapted from Cork and Krueger, *Adv. Appl. Microbiol.* 36:1–66, 1991.

Plasmids with the same name encode a similar degradative pathway even though they have different sizes and were described in different laboratories. 2,4-D, 2,4-dichlorophenoxyacetic acid.

BACTERIA CAN BE Engineered to degrade Several Different "toxic" Compounds

Figure 13.5 Schematic representation of the development of a bacterial strain that can degrade camphor, octane, xylene, and naphthalene. Strain A, which contains a CAM (camphor-degrading) plasmid, is mated with strain B, which carries an OCT (octane-degrading) plasmid. Following plasmid transfer and homologous recombination between the two plasmids, strain E carries a CAM and OCT biodegradative fusion plasmid. Strain C, which contains a XYL (xylene-degrading) plasmid, is mated with strain D, which contains a NAH (naphthalene-degrading) plasmid, to form strain F, which carries both of these plasmids. Finally, strain E and strain F are mated to yield strain G, which carries the CAM/OCT fusion plasmid, the XYL plasmid, and the NAH plasmid.

PSEUDOMONAS



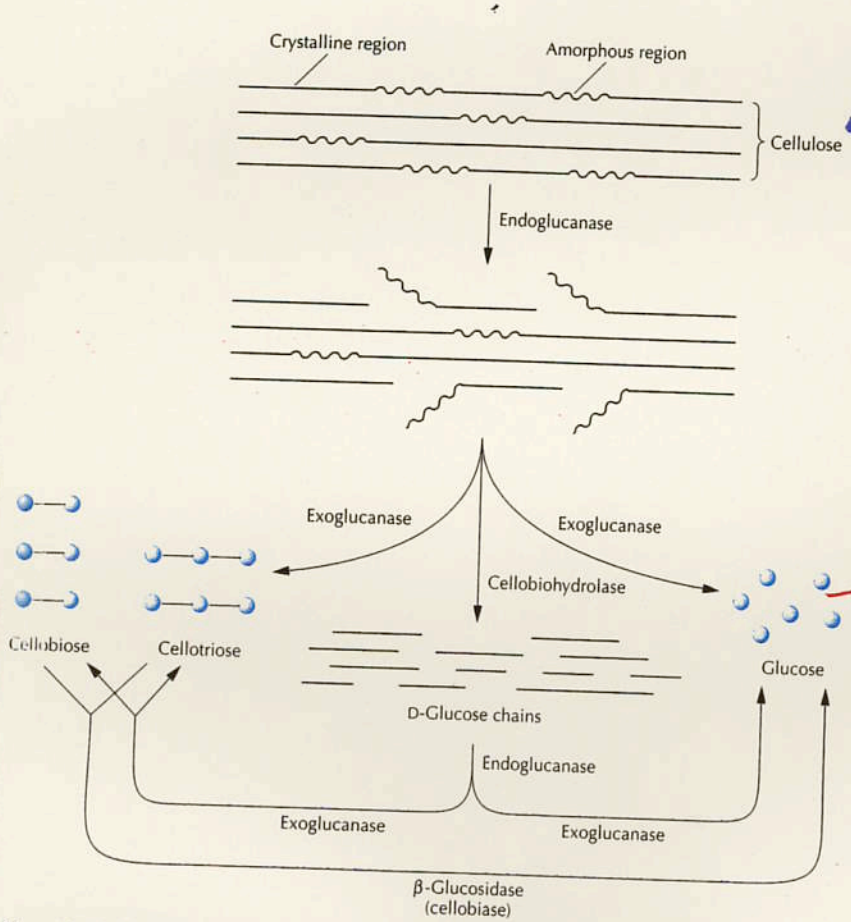
A LANDMARK DECISION

CHAKRABARTY US PATENT 4,259,444 1981
genetically engineered MICROORGANISMS ARE "inventions"

LIFE CAN BE PATENTED!

BACTERIA CAN BE Engineered To degrade BIOMASS WASTE Products

WASTE containing cellulose



green waste!

Energy for Bacteria

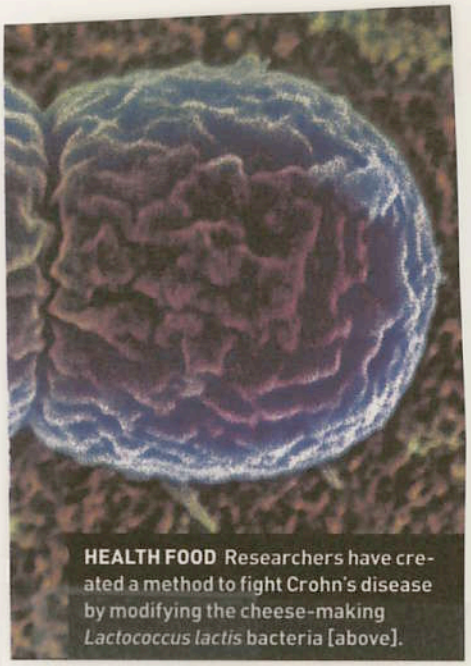
Figure 13.15 Enzymatic biodegradation of cellulose. Cellulose hydrolysis begins with the cleavage of β-1,4-linkages within the accessible amorphous regions of the cellulose chains by endoglucanase(s). This reaction is followed by the removal of oligosaccharides from the reducing ends of the partially cleaved cellulose chains by exoglucanase(s) and cellobiohydrolase(s). The degradation of cellulose is completed when the cellobiose and cellotriose are converted to glucose by β-glucosidase.

(+) ENERGY!

Metagenomics

Agriculture, Timber Processing, Human Activities:
e.g.) plants left after harvests, animal manure with grasses, municipal waste paper, cotton left-overs, hay, etc

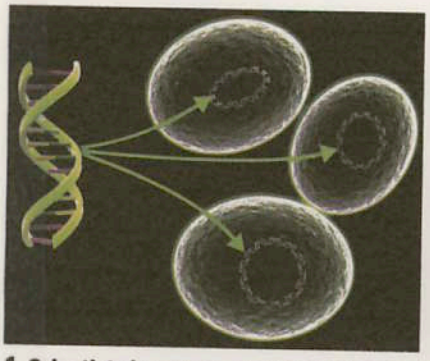
USING BACTERIA TO DELIVER PROTEIN TO YOUR BODY TO TREAT DISEASE



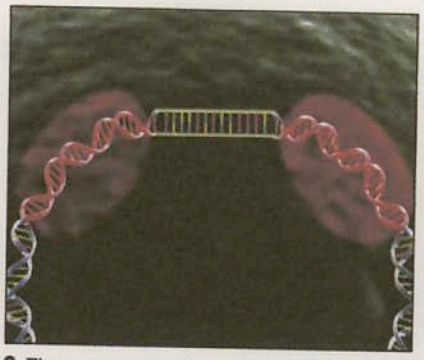
HEALTH FOOD Researchers have created a method to fight Crohn's disease by modifying the cheese-making *Lactococcus lactis* bacteria [above].

THE MICROBIAL ATTACK PLAN

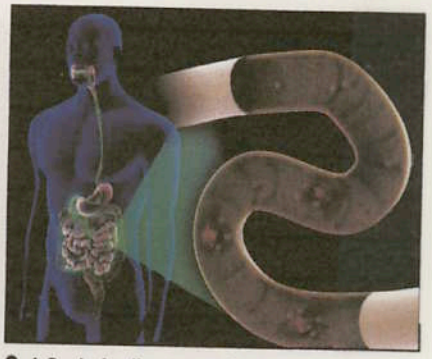
The painful ulcers of Crohn's disease occur when the body's immune system attacks the normal bacteria found in the gut. Now scientists are using microbes to fight back



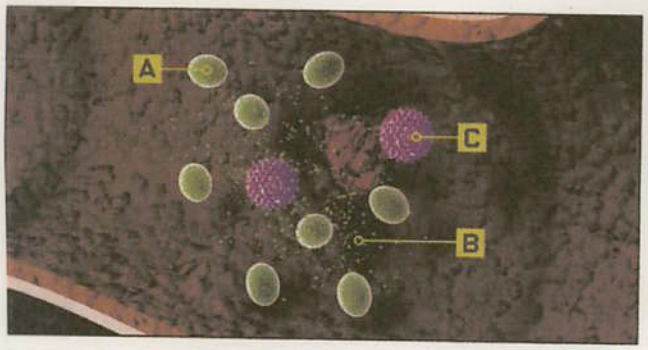
1. Scientists insert a gene that produces interleukin-10 (IL-10), an immune-calming molecule, into the *L. lactis* bacteria.



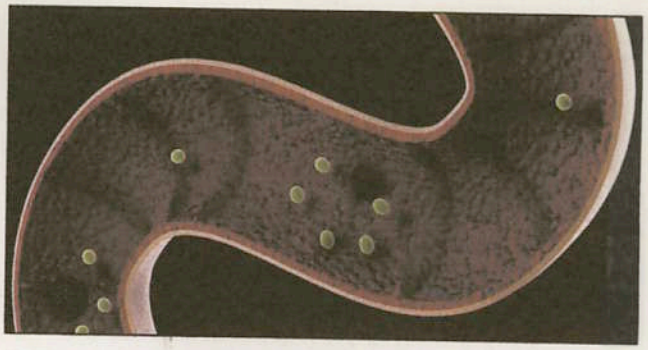
2. The new gene replaces the gene that produces thymide, an essential nutrient, so **the modified bacteria can live for only a few days.**



3. A Crohn's-disease patient swallows the modified bacteria, **delivering the IL-10 payload straight into the intestines.**



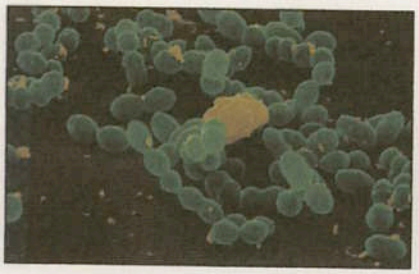
4. The bacteria [A] colonize the gut and begin producing IL-10 [B]. **This compound inhibits the body's natural killer cells** [C] and suppresses the patient's overactive immune response.

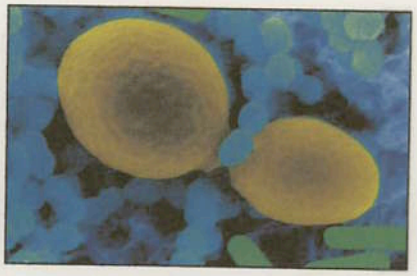


5. As long as the patient keeps ingesting the modified *L. lactis* bacteria, **the bacteria will produce enough IL-10 to stop the immune system from attacking the intestines,** giving the ulcers time to heal.

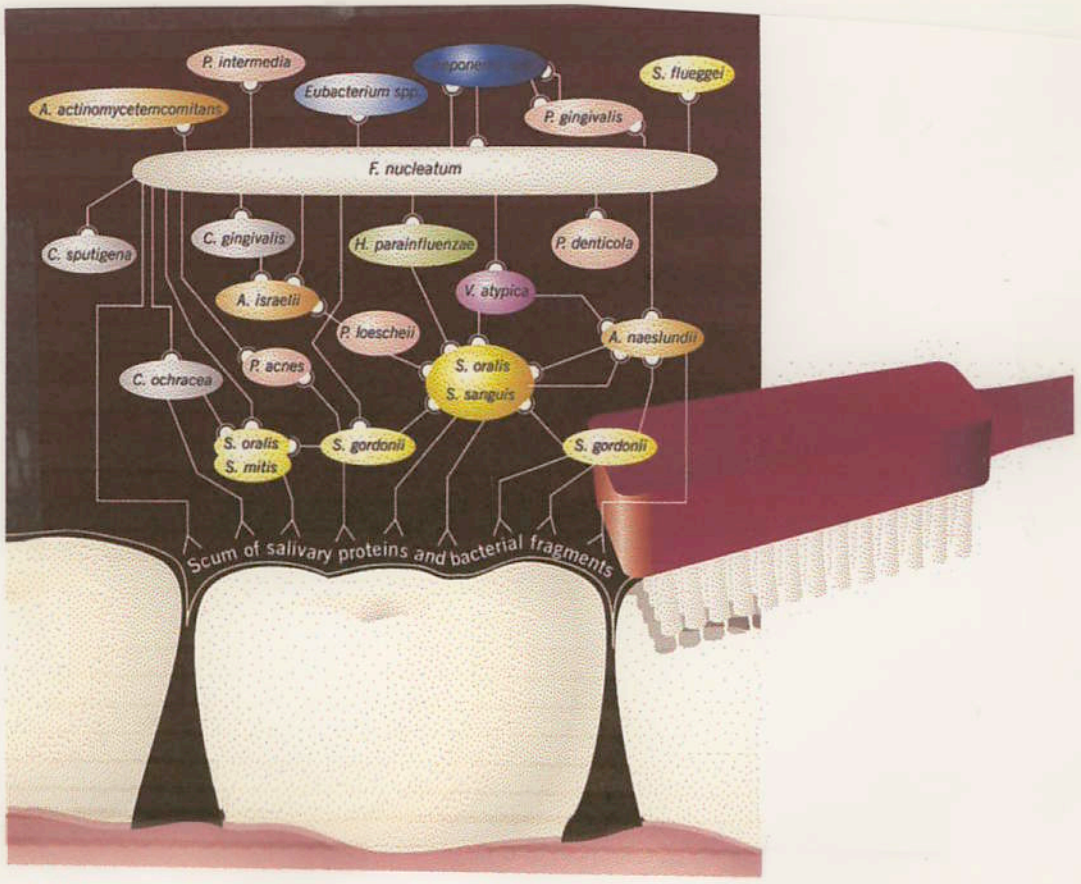
USING ENGINEERED BACTERIA TO FIGHT TOOTH DECAY

"FORTUNATELY, WE HAD NO IDEA WHAT WAS AHEAD."—JEFFREY HILLMAN





WAITING ROOM Jeffrey Hillman [facing page] developed a strain of *S. mutans* tooth bacteria [above left] that doesn't produce enamel-eroding acid. Ordinary *S. mutans* [above right, in blue] lives in the mouth along with yeast [yellow] and *P. gingivalis* bacteria [green].



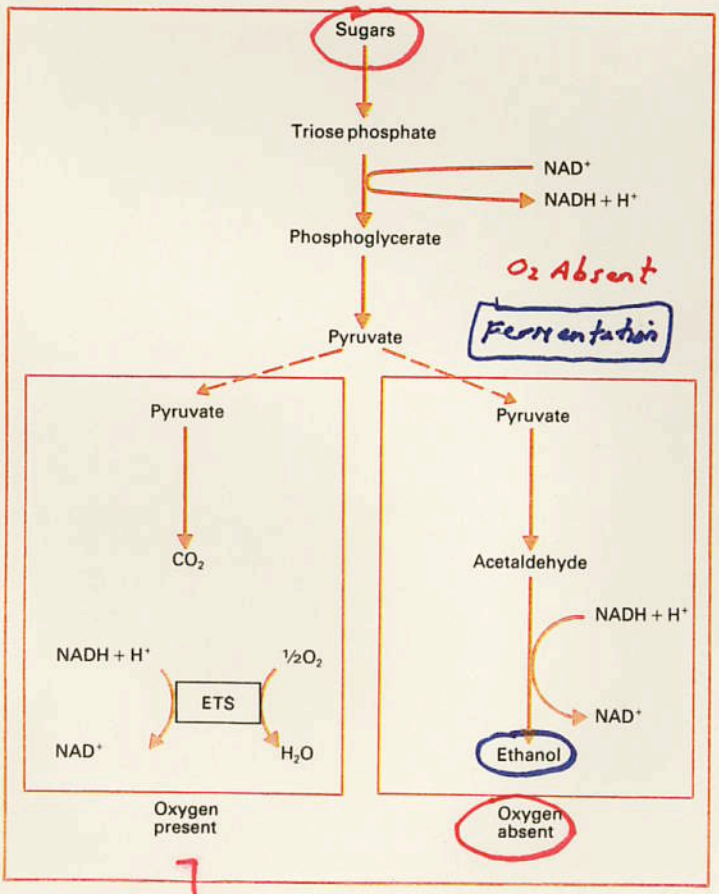
GENETIC ENGINEERING
Yeasts

USING YEAST AS FACTORIES AND "CATALYSTS"

Table 36.1 Fungi

Phylum	Typical Examples		Key Characteristics	Approximate Number of Living Species
Ascomycota	Yeasts, truffles, morels		Develop by sexual means; ascospores are formed inside a sac called an ascus; asexual reproduction is also common	32,000
Imperfect fungi	*Aspergillus*, *Penicillium*		Sexual reproduction has not been observed; most are thought to be ascomycetes that have lost the ability to reproduce sexually	17,000
Basidiomycota	Mushrooms, toadstools, rusts		Develop by sexual means; basidiospores are borne on club-shaped structures called basidia; the terminal hyphal cell that produces spores is called a basidium; asexual reproduction occurs occasionally	22,000
Zygomycota	*Rhizopus* (black bread mold)		Develop sexually and asexually; multinucleate hyphae lack septa, except for reproductive structures; fusion of hyphae leads directly to formation of a zygote, in which meiosis occurs just before it germinates	1050

What are fungi & yeasts?



Energy (ATP)

USING YEAST TO MAKE RECOMBINANT PROTEINS

VACCINES
- Hepatitis B virus surface antigen
- Malaria circumsporozoite protein
- HIV-1 envelope protein

DIAGNOSTICS
- Hepatitis C virus protein
- HIV-1 antigens

HUMAN THERAPEUTIC AGENTS
- Epidermal growth factor
- Insulin
- Insulin-like growth factor
- Platelet-derived growth factor
- Proinsulin
- Fibroblast growth factor
- Granulocyte-macrophage colony-stimulating factor
- α_1 antitrypsin
- Blood coagulation factor XIIIa
- Hirudin
- Human growth factor
- Human serum albumin

Advantages over Bacteria?

Vectors?
Switches?

USING YEAST TO MAKE ALCOHOLIC BEVERAGES

Table 6.5 The origins of the different kinds of alcoholic beverages.

Alcoholic beverage	Origin
Non-distilled	
Beer	On germination, starch in barley grains is converted to sugar, which is extracted by boiling in water to produce wort and this is fermented
Cider	Fermentation of apple juice
Wine	Fermentation of grape juice
Sake	Starch in steamed rice is hydrolysed with *Aspergillus oryzae* and the sugars released are fermented with yeast
Distilled	
Whisky (Scotch)	Distillation of alcohol produced from barley
Whiskey—Irish	Pot still whiskey produced from alcohol derived from a mixture of barley, wheat and rye. Grain whiskey produced from alcohol derived from maize
—Rye	Produced from alcohol derived from rye
—Bourbon	Produced from alcohol derived from maize
Rum	Distillation of fermented molasses, a by-product of sugar cane refining
Vodka	Distillation of alcohol produced from any non-grain carbohydrate source, e.g. potatoes
Gin	Distillation of alcohol derived from maize or rye and redistillation in presence of herbs and juniper berries
Tequila	Distillation of fermented extracts of Mexican cactus

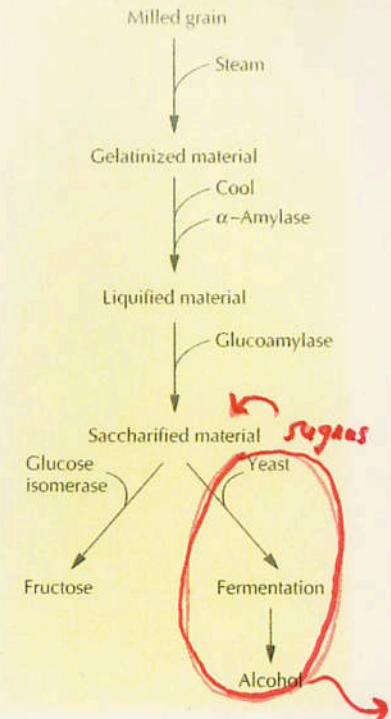


Figure 13.10 Industrial production of fructose and alcohol from starch.

CH_3CH_2OH

Cannot grow above 18% ethanol!
Some can grow to 25% (Fuel Production)

ANAEROBIC FERMENTATION BY yeasts

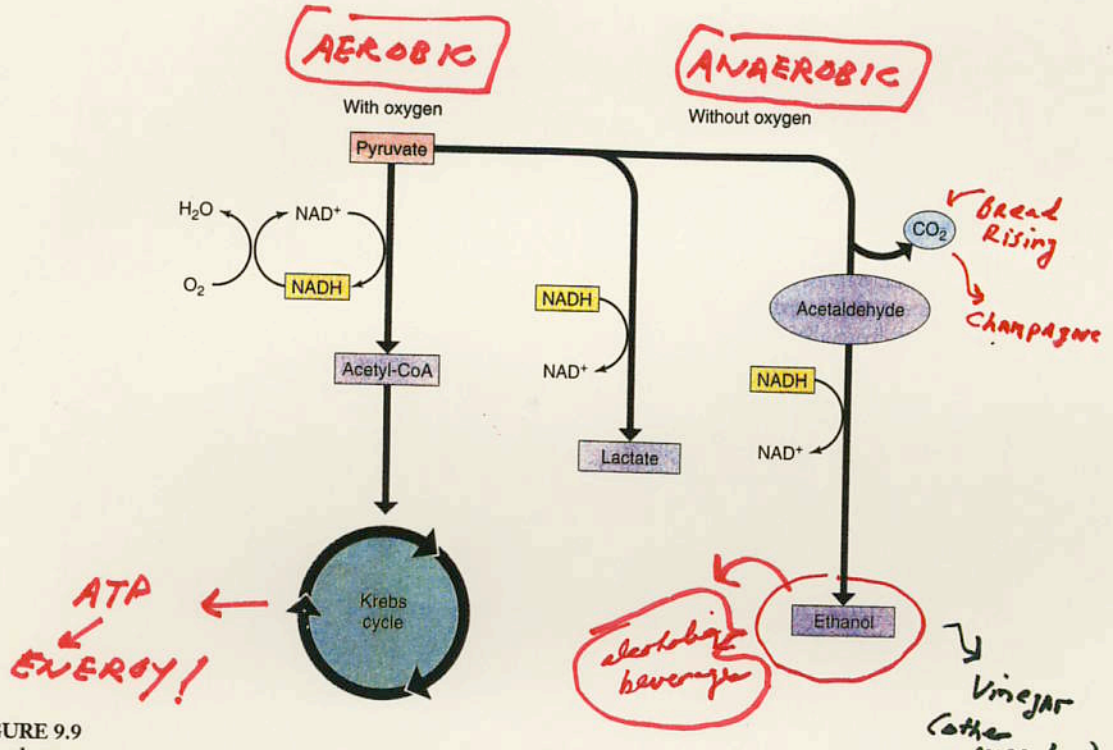


FIGURE 9.9
What happens to pyruvate, the product of glycolysis? In the presence of oxygen, pyruvate is oxidized to acetyl-CoA, which enters the Krebs cycle. In the absence of oxygen, pyruvate is instead reduced, accepting the electrons extracted during glycolysis and carried by NADH. When pyruvate is reduced directly, as in muscle cells, the product is lactate. When CO_2 is first removed from pyruvate and the product, acetaldehyde, is then reduced, as in yeast cells, the product is ethanol.

Yeasts could be Genetically Engineered to Enhance Alcohol Production

Enhance production, alter flavors, remove "off" flavors!

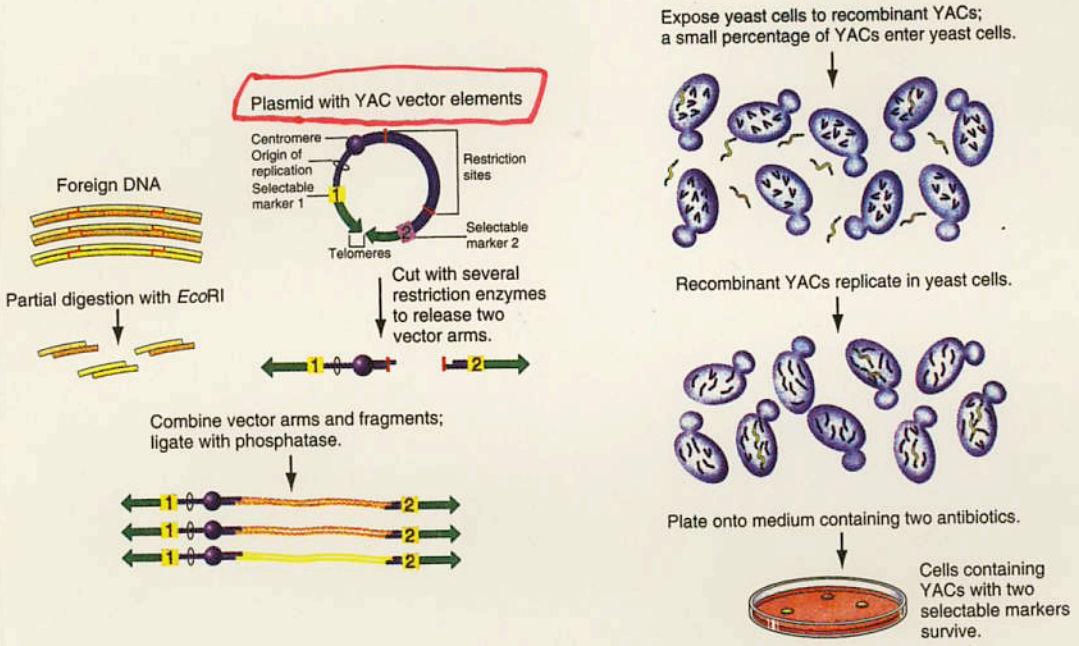


Figure 8.7 YAC vectors take advantage of DNA elements used for normal chromosome segregation within yeast cells. Two distinct arms make up each YAC vector. At the end of one arm is a telomere followed by a selectable marker, then a centromere, and finally a restriction site. The second arm lacks a centromere but has a telomere at one end, a restriction site at the other, and a second selectable marker in the middle. One of the two arms must also contain a yeast origin of replication. To make YAC-insert recombinants, you cut the two YAC arms and large foreign genomic fragments with the same restriction enzyme, mix the YAC arms with the foreign restriction fragments, and treat the mixture with phosphatase. As with bacteria exposed to plasmids, a small percentage of yeast cells exposed to YAC-insert recombinants will take up the recombinant molecules. And like bacteria that harbor plasmid vectors, yeast cells transformed by properly constructed recombinant YACs containing two selectable markers will survive and propagate in a medium infused with two antibiotics. Yeast cells with one or no marker will not. The properly constructed YAC recombinants will replicate and be transmitted along with other chromosomes inside the surviving yeast cells. Such proper YACs must meet three requirements: (1) They must contain an insert; (2) they must carry one—and only one—centromere, since those with more than one centromere will not segregate properly during mitosis; and (3) they must have a telomere at both ends. Tips without a telomere will fuse with another chromosome or decay. Since only those recombinants composed of two different arms flanking an insert will satisfy these requirements, the ability to segregate properly after replication ensures the reproduction of mostly single vector–single insert recombinants.

What is a YAC?

Haven't yet Why?

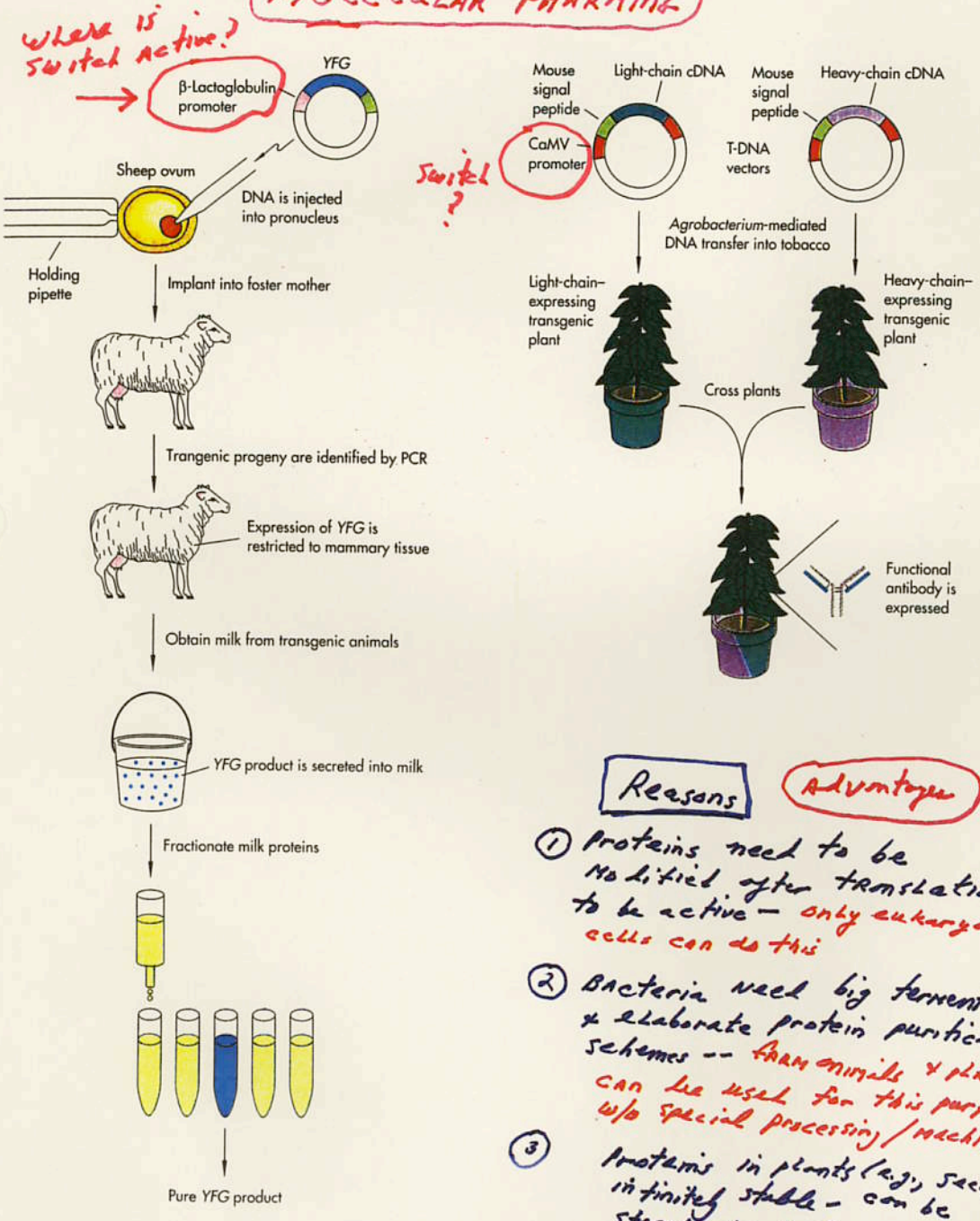
? function ?

GENETIC ENGINEERING

ANIMALS & PLANTS

Animals & Plants can also be used as factories to produce large amounts of Human Proteins

MOLECULAR PHARMING



Reasons — **Advantages**

1. Proteins need to be modified after translation to be active — only eukaryotic cells can do this — **MODifications**
2. Bacteria need big fermenters & elaborate protein purification schemes -- transgenic animals & plants can be used for this purpose w/o special processing / machinary — **COST**
3. Proteins in plants (e.g., seeds) in tissues stable - can be stored cheaply (& grown cheaply) for long periods of time! — **STABILITY**

MAKING RECOMBINANT HUMAN PROTEINS IN ANIMALS

Table 19.3 Some exogenous proteins that have been expressed in the mammary glands of transgenic animals

Antithrombin III
Calcitonin
Erythropoietin
Factor IX
Factor VIII
Fibrinogen
Glucagon-like peptide
Granulocyte colony-stimulating factor
Growth hormone
Hemoglobin
Human serum albumin
Insulin
Insulin-like growth factor 1
Interleukin 2
Lactoferrin
Lysozyme
Monclonal antibodies
Nerve growth factor β
Protein C
Superoxide dismutase
Tissue plasminogen activator
α1-Antitrypsin
α-Glucosidase
α-Lactalbumin

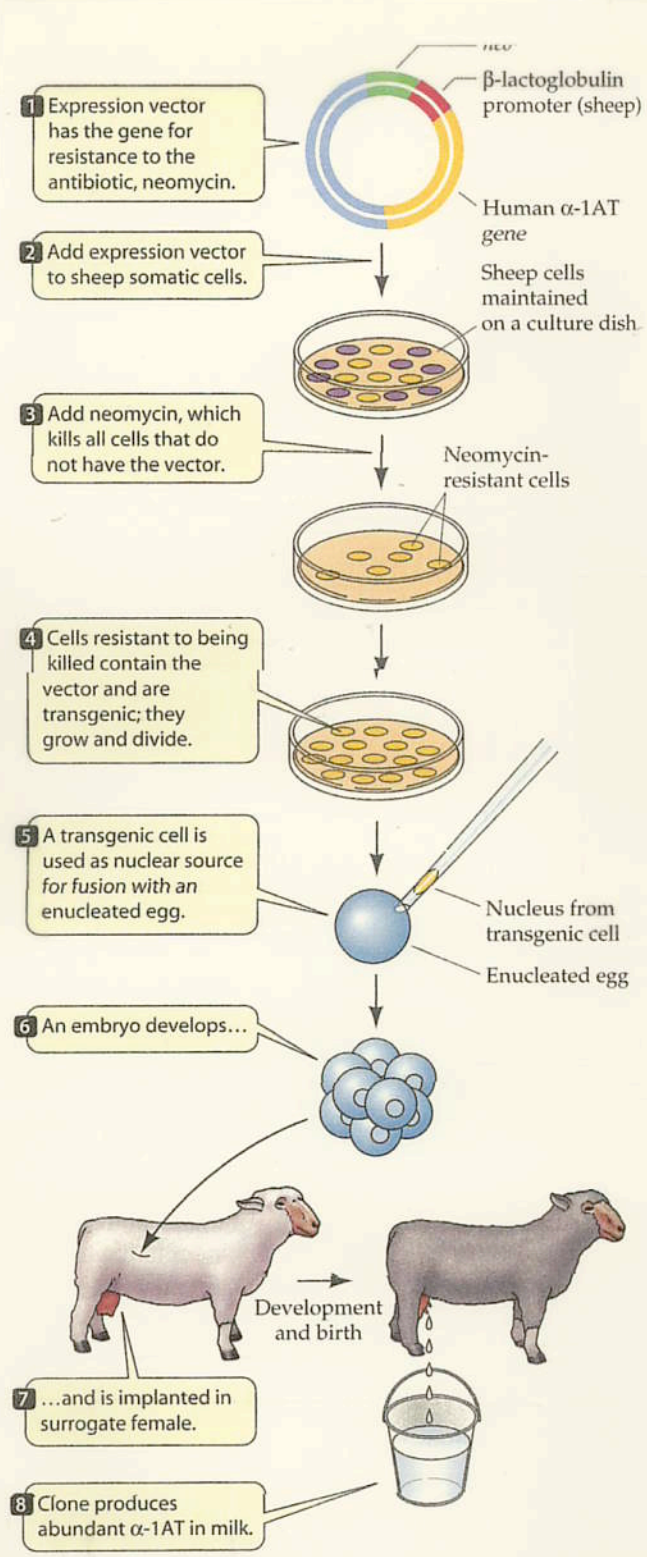
Advantages over bacteria?

Table 19.2 Milk production and estimated recombinant protein yields from organisms used for the expression of transgenes in mammary glands

Organism	Annual milk yield (liters)	Estimated recombinant protein per female (kg/yr)
Rabbit	5	0.02
Pig	300	1.5
Sheep	500	2.5
Goat	900	4
Cow	10,000	60 !!!

advantages?

USING CLONING & RECOMBINANT DNA TO MAKE TRANSGENIC PHARM ANIMALS



17.15 Production of Transgenic Clones for "Pharming"
The production of transgenic animals involves a combination of DNA technology and reproductive technology.

Other TRANSGENIC ANIMALS Have Been Created

TABLE 2.1 State of the art of transgenic technology for selected organisms.

Organism	Transfection	Viral vectors	Transposon	ES cells	Nuclear transfer
Mouse	4[a]	2	1	4[a]	2
Cow	3	1	0	0	2
Sheep	3	0	0	0	2
Goat	3	0	0	0	2
Pig	3	0	0	0	2
Rabbit	3	0	0	1	0
Chicken	1	2	1	0	0
Altlantic salmon	3	0	0	0	0
Channel catfish	2	0	0	0	0
Tilapia	3	0	0	0	0
Zebrafish	1	0	0	1	1
Crustaceans	1	1	0	0	0
Mollusks	1	1	0	0	0
Drosophila	2	2	2	2	0
Mosquito	1	0	2	0	0

NOTE: 0: No significant progress.
1: Has been accomplished experimentally (proof of concept).
2: Routine experimental use.
3: Commercialization sought.
4: Widespread production.
[a] For experimental uses.
See (Dove, 2000)

TRANSGENIC SALMON

control

superfish

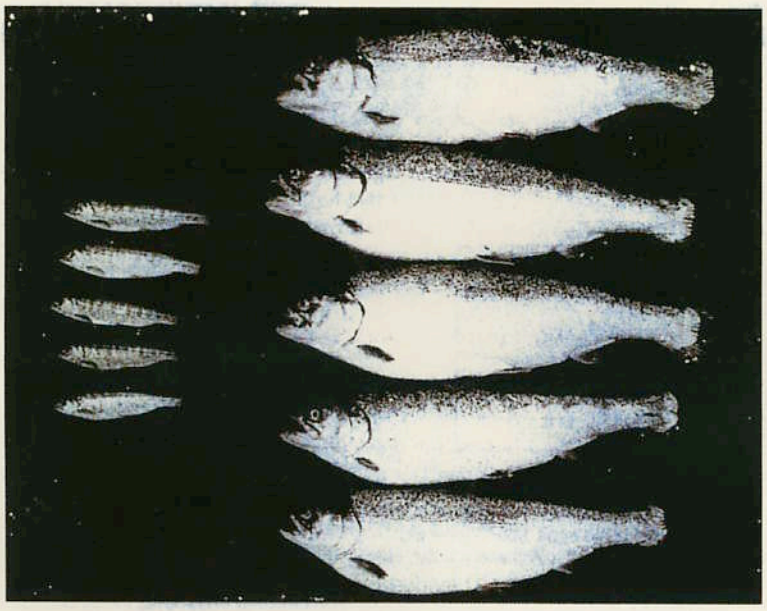


Figure 8.11 Comparison of 1-month-old coho salmon siblings; nonengineered fish are at left, transgenic fish are at right. The largest fish (top right) is 41.8 cm in length.

GROWTH HORMONE GENE

What are the issues with these fish?

"Pharming" in Plants

NICOTIANA BENTHAMIANA, a tobacco plant, serves as a biofactory for producing antibodies against cancer.

Advantages
1. Cost
2. Simplicity of method
3. Stability of proteins etc.

Table 14.5 A selection of pharmaceutical recombinant human proteins expressed in plant systems.

Species	Recombinant human product	Reference
Tobacco, sunflower (plants)	Growth hormone	Barta *et al.* 1986
Tobacco, potato (plants)	Serum albumin	Sijmons *et al.* 1990
Tobacco (plants)	Epidermal growth factor	Higo *et al.* 1993
Rice (plants)	α-Interferon	Zhu *et al.* 1994
Tobacco (cell culture)	Erythropoietin	Matsumoto *et al.* 1995
Tobacco (plants)	Haemoglobin	Dieryck *et al.* 1997
Tobacco (cell culture)	Interleukins-2 and 4	Magnuson *et al.* 1998
Tobacco (root culture)	Placental alkaline phosphatase	Borisjuk *et al.* 1999
Rice (cell culture)	α_1-Antitrypsin	Terashima *et al.* 1999
Tobacco (seeds)	Growth hormone	Leite *et al.* 2000
Tobacco (chloroplasts)	Growth hormone	Staub *et al.* 2000

Antigen	Host-plant system	Reference
Herpes virus B surface antigen	Tobacco	Mason *et al.* 1992
Rabies glycoprotein	Tomato	McGarvey *et al.* 1995
Norwark virus coat protein	Tobacco, potato	Mason *et al.* 1996
Foot-and-mouth virus VP1	*Arabidopsis*	Carrillo *et al.* 1998
Cholera toxin B subunit	Potato	Arakawa *et al.* 1998
Human cytomegalovirus glycoprotein B	Tobacco	Tackaberry *et al.* 1999

Vaccines

Table 14.7 A selection of recombinant vaccines against animal viruses produced in plants.

RE-ENGINEERING PLANTS AS DRUG FACTORIES

BACK TO THE FUTURE

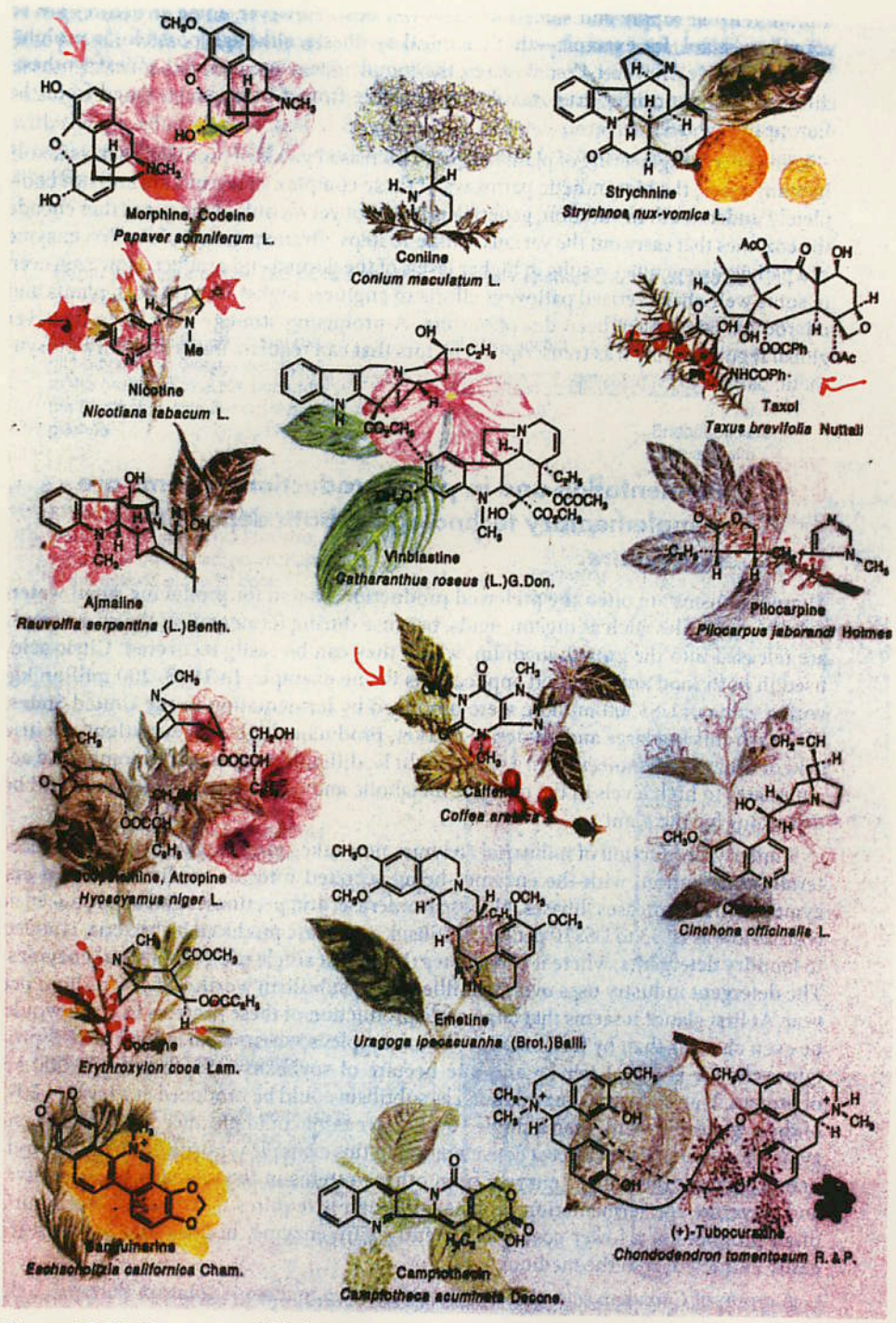
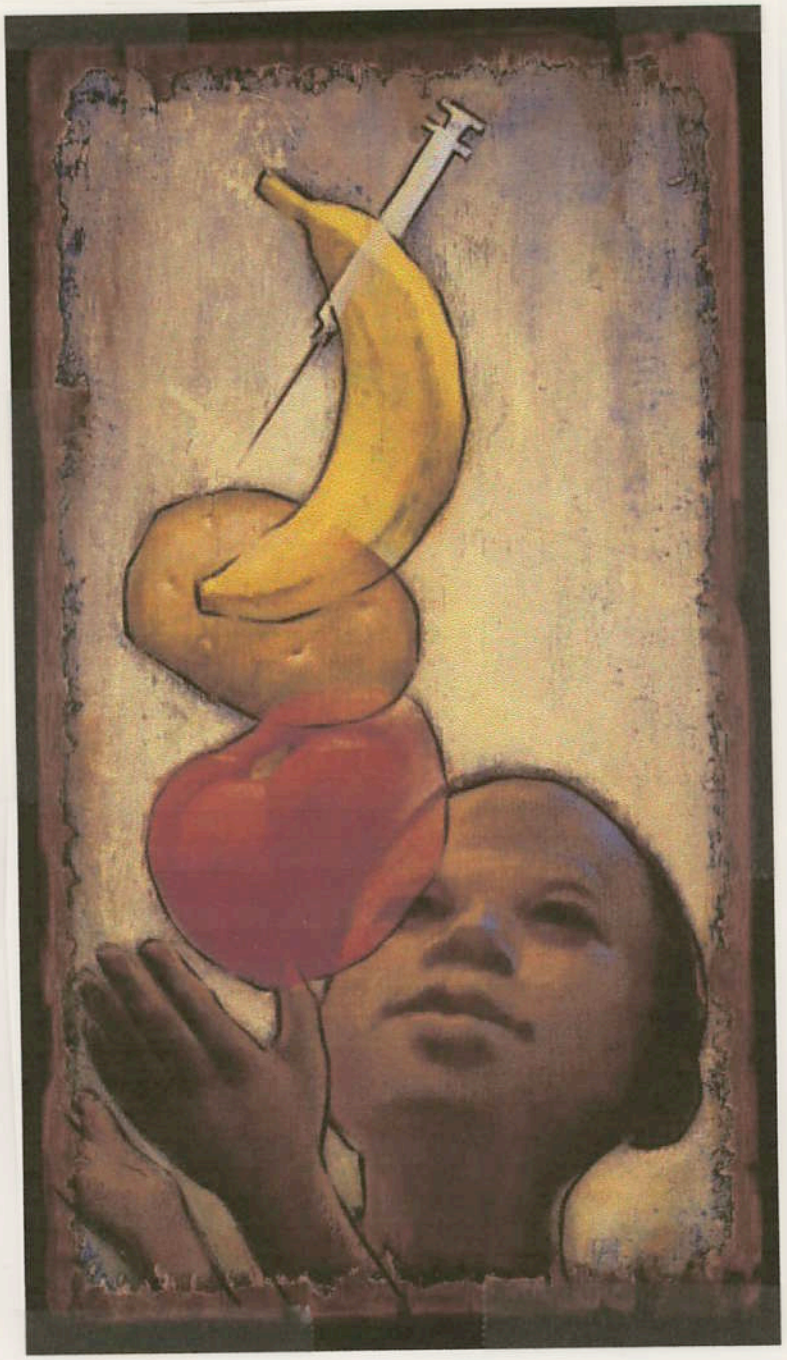


Figure 19.11 Structures of biologically active alkaloids and the plants that produce them. *Source:* Kutchan, T. M. 1995. Alkaloid biosynthesis—The basis for metabolic engineering of medicinal plants. *Plant Cell* 7:1059–1070.

One day children may get immunized by munching on foods instead of enduring shots. More important, food vaccines might save millions who now die for lack of access to traditional inoculants

Edible Vaccines

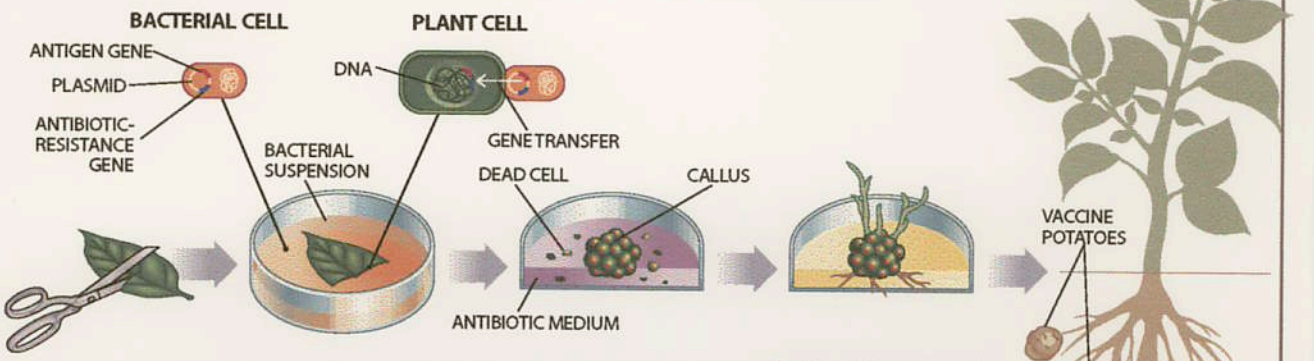
by William H. R. Langridge



46

HOW TO MAKE AN EDIBLE VACCINE

One way of generating edible vaccines relies on the bacterium *Agrobacterium tumefaciens* to deliver into plant cells the genetic blueprints for viral or bacterial "antigens"—proteins that elicit a targeted immune response in the recipient. The diagram illustrates the production of vaccine potatoes.



1 Cut leaf.

2 Expose leaf to bacteria carrying an antigen gene and an antibiotic-resistance gene. Allow bacteria to deliver the genes into leaf cells.

3 Expose leaf to an antibiotic to kill cells that lack the new genes. Wait for surviving (gene-altered) cells to multiply and form a clump (callus).

4 Allow callus to sprout shoots and roots.

5 Put in soil. Within three months, the plantlets will grow into plants bearing antigen-laden vaccine potatoes.

JARED SCHNEIDMAN DESIGN

Table 1. Edible transgenic plant vaccines

Vaccine	Edible plant	Ref.
Norwalk virus particle	Potato	3
	Tomato	4
Heat-labile enterotoxin B subunit	Potato	5
	Maize	6
	Soybean	20
Cholera toxin B subunit	Rice	14
	Potato	21
Enterotoxigenic *Escherichia coli* fimbrial subunit	Soybean	11
Japanese cedar pollen peptide	Rice	19

HUMAN GENETIC ENGINEERING

21.4 Principles of gene therapy

Gene therapy involves the direct genetic modification of cells of the patient in order to achieve a therapeutic goal. There are basic distinctions in the types of cells modified, and the type of modification effected.

- **Germ-line gene therapy** produces a permanent transmissible modification. This might be achieved by modification of a gamete, a zygote or an early embryo. Germ-line therapy is banned in many countries for ethical reasons (see *Ethics Box 2*).
- **Somatic cell gene therapy** aims to modify specific cells or tissues of the patient in a way that is confined to that patient. All current gene therapy trials and protocols are for somatic cell therapy.

Somatic cells might be modified in a number of different ways (*Figure 21.4*).

- **Gene supplementation** (also called gene augmentation) aims to supply a functioning copy of a defective gene. This would be used to treat loss-of-function conditions (Section 16.4) where the disease process is the result of a gene not functioning here and now. Cystic fibrosis would be a typical candidate. It would not be suitable for loss-of-function conditions where irreversible damage has already been done, for example through some failure in embryonic development. Cancer therapy could involve gene supplementation to increase the immune response against a tumor or to replace a defective tumor suppressor gene.
- **Gene replacement** is more ambitious: the aim is to replace a mutant gene by a correctly functioning copy, or to correct a mutation *in situ*. Gene replacement would be required for gain-of-function diseases where the resident mutant gene is doing something positively bad.
- **Targeted inhibition of gene expression** is especially relevant in infectious disease, where essential functions of the pathogen are targeted. It could also be used to silence activated oncogenes in cancer, to damp down unwanted responses in autoimmune disease and maybe to silence a gain-of-function mutant allele in inherited disease.
- **Targeted killing of specific cells** is particularly applicable to cancer treatment.

Expectations about gene therapy have followed a manic-depressive course over the past 15 years as cycles of over-optimism are followed by bouts of excessive pessimism. An important report for the US National Institutes of Health in 1995 attempted to inject some reality (*Box 21.1*). Since then

IN VIVO CYSTIC FIBROSIS GENE THERAPY

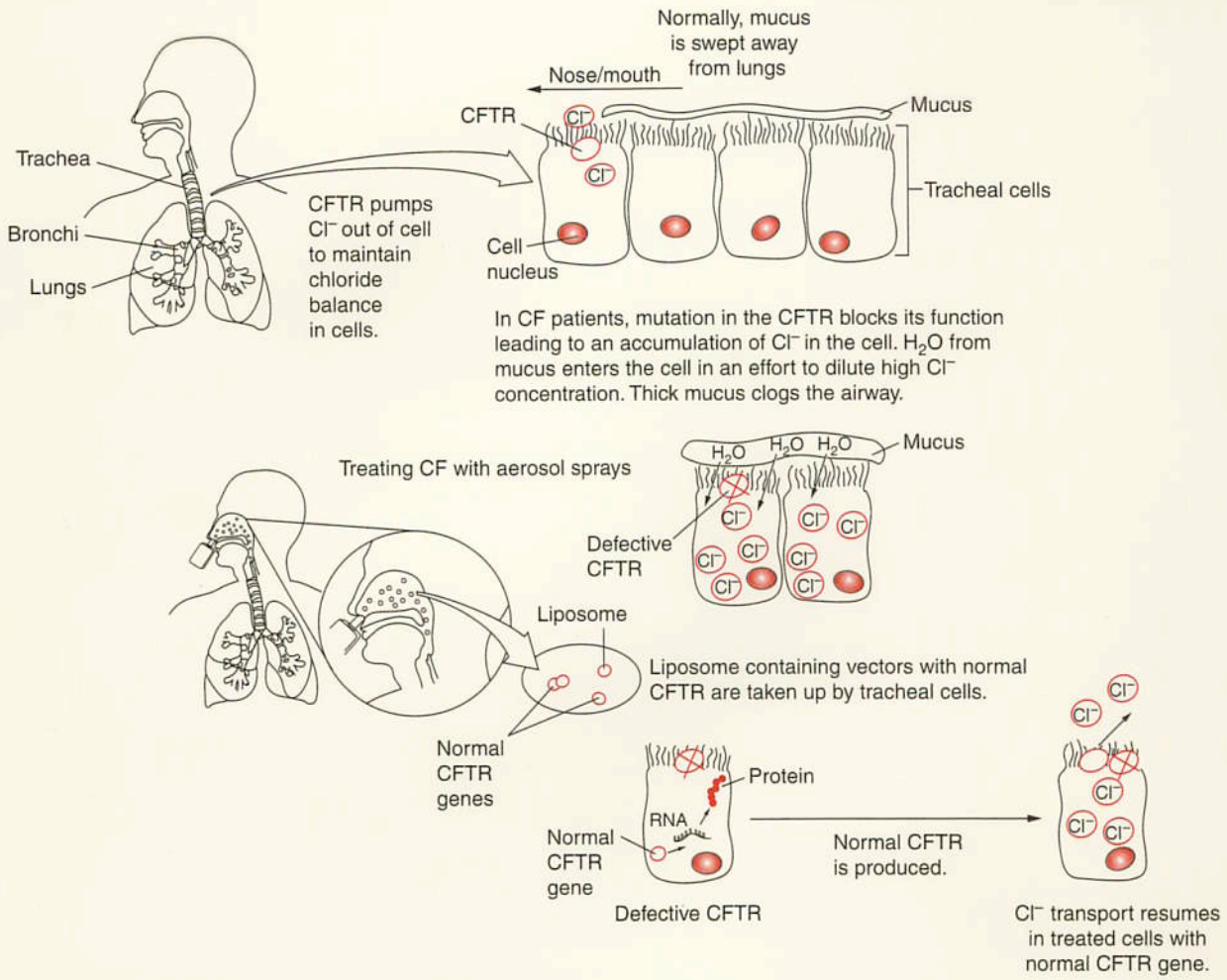


Figure 11.13 Treating Cystic Fibrosis by Gene Therapy

Gene Therapy Research Offers Promise of a Cure for Cystic Fibrosis

Gene therapy offers great promise for life-saving treatment for CF patients since it targets the cause of CF rather than just treating symptoms. Gene therapy for CF had its start in 1990, when scientists successfully corrected faulty CFTR genes by adding normal copies of the gene to laboratory cell cultures.

In 1993, the first experimental gene therapy treatment was given to a patient with CF. Researchers modified a common cold virus to act as a delivery vehicle - or "vector"- carrying the normal genes to the CFTR cells in the airways of the lung.

Subsequent studies have tested other methods of gene delivery, such as fat capsules, synthetic vectors, nose drops or drizzling cells down a flexible tube to CFTR cells lining the airways of lungs. Researchers are now testing aerosol delivery using nebulizers.

But finding the best delivery system for transporting normal CFTR genes is only one problem that scientists must solve to develop an effective treatment for CF. Scientists must also determine the life span of affected lung cells, identify the "parent cells" that produce CFTR cells, find out how long treatment should last and how often it needs to be repeated.

The first cystic fibrosis gene therapy experiments have involved lung cells because these cells are readily accessible and because lung damage is the most common, life-threatening problem in CF patients. But scientists hope that the technologies being developed for lung cells will be adapted to treat other organs affected by CF.

IN VIVO CANCER SUICIDE CELL GENE THERAPY

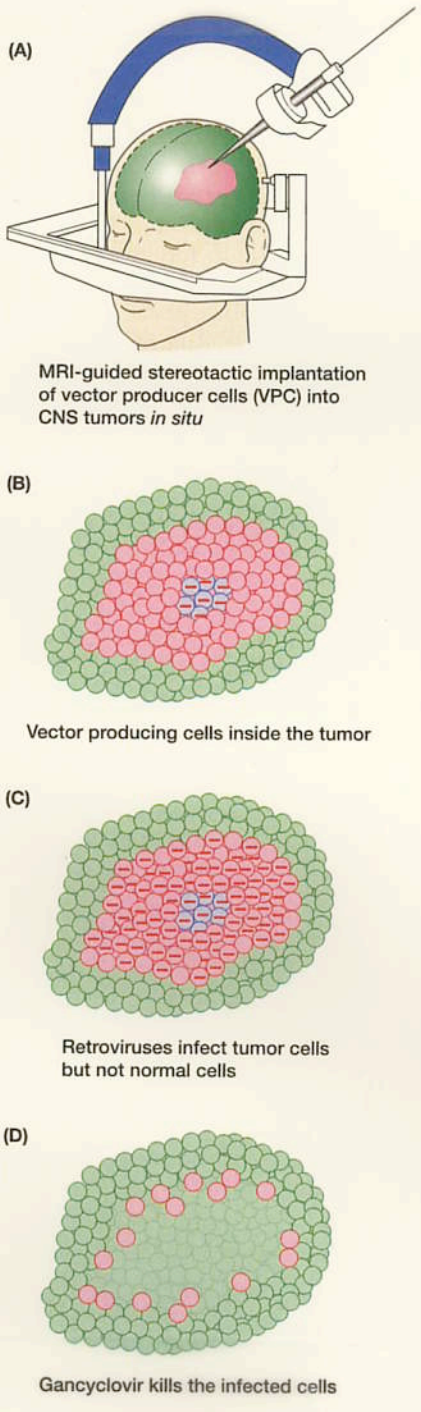
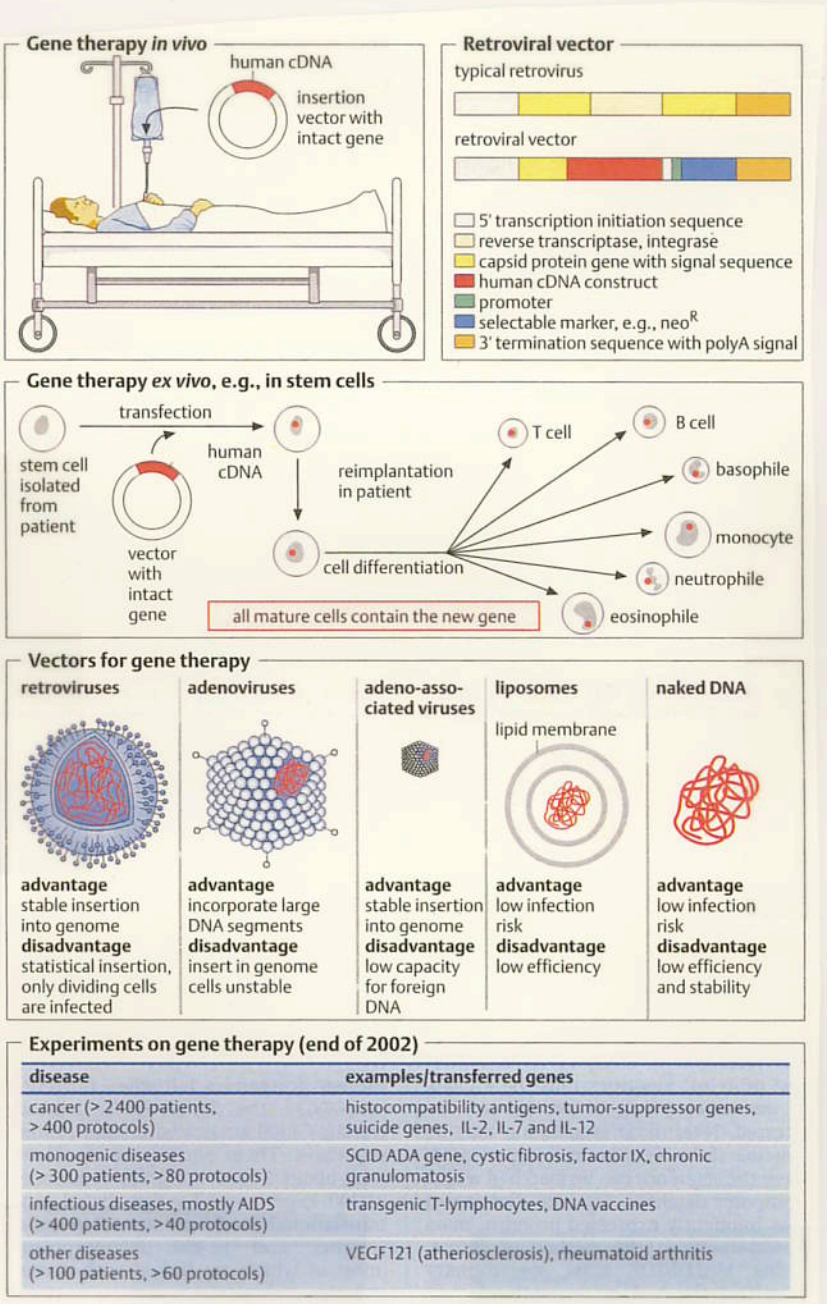


Figure 21.12: *In vivo* gene therapy for brain tumors.

A retrovirus is engineered to produce the herpes simplex virus thymidine kinase (HSV-TK). Vector-producing cells (VPC; blue) are injected into the brain tumor. Because retroviruses infect only dividing cells, they infect the tumor cells (pink) but not the surrounding normal brain tissue (green). The nontoxic prodrug gancyclovir (gcv) is given intravenously. In TK^+ cells gcv is converted to the highly toxic gcv-triphosphate and the cell is killed.

EX VIVO VS. IN VIVO SOMATIC CELL GENE THERAPY

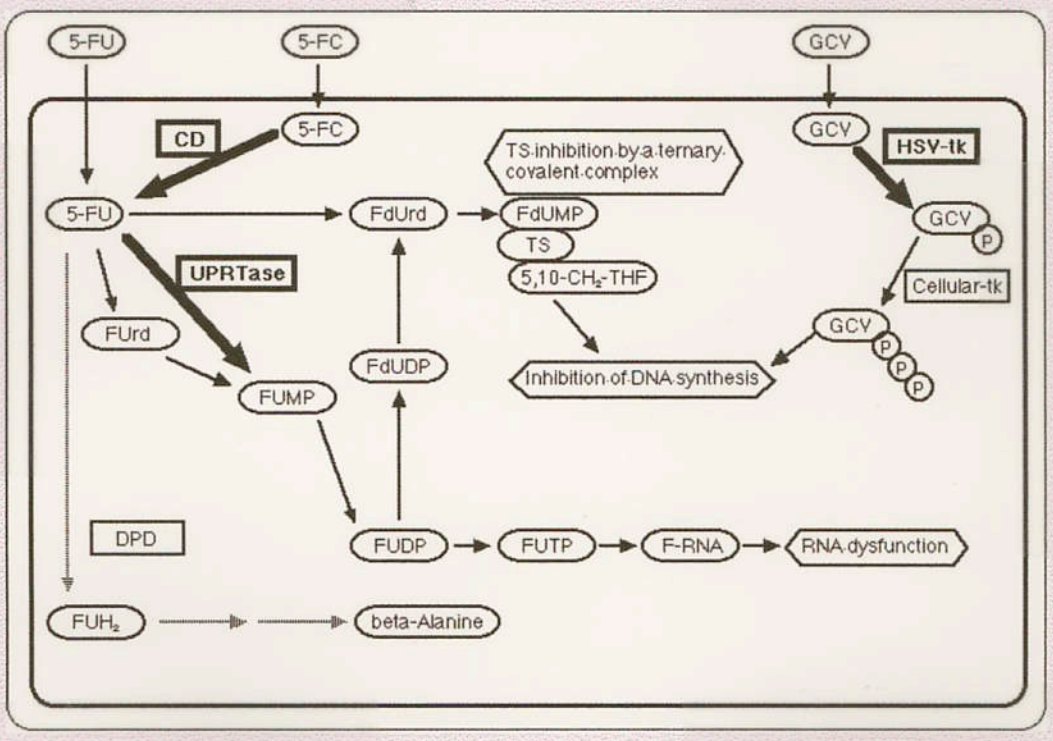


Experiments on gene therapy (end of 2002)

disease	examples/transferred genes
cancer (> 2 400 patients, > 400 protocols)	histocompatibility antigens, tumor-suppressor genes, suicide genes, IL-2, IL-7 and IL-12
monogenic diseases (> 300 patients, > 80 protocols)	SCID ADA gene, cystic fibrosis, factor IX, chronic granulomatosis
infectious diseases, mostly AIDS (> 400 patients, > 40 protocols)	transgenic T-lymphocytes, DNA vaccines
other diseases (> 100 patients, > 60 protocols)	VEGF121 (atheriosclerosis), rheumatoid arthritis

SUICIDE GENE THERAPY

Gene Set Bank - Suicide gene therapy



Neurosurgical Focus

Journal of Neurosurgery
Table of Contents
Feburary 2000

Treatment of progressive or recurrent pediatric malignant supratentorial brain tumors with herpes simplex virus thymidine kinase gene vector--producer cells followed by intravenous ganciclovir administration

Roger J. Packer, M.D., Cory Raffel, M.D., Ph.D., Judith G. Villablanca, M.D., Jörg-Christian Tonn, M.D., Stefan E. Burdach, M.D., Klaus Burger, M.D., Ph.D., Deborah LaFond, P.N.P., J. Gordon McComb, M.D., Philip H. Cogen, M.D., Ph.D., Gilbert Vezina, M.D., and Leonard P. Kapcala, M.D.

Departments of Neurology, Pediatrics, Hematology/Oncology, Neurosurgery, and Diagnostic Imaging, Children's National Medical Center, Washington, D.C.; The George Washington University Hospital, Washington, D.C.; Department of Neurosurgery, Mayo Clinic, Rochester, Minnesota; Departments of Pediatrics and Neurosurgery, Children's Hospital Los Angeles and University of Southern California, Los Angeles, California; Kinderklinik, Würzburg, Germany; Universitäts-Kinderklinik, Düsseldorf, Germany; Department of Pediatrics, Martin-Luther Universität Halle--Wittenberg, Halle, Germany; Novartis Pharma GmbH, Nuremberg, Germany; and Genetic Therapy, Inc., Bethesda, Maryland

Object. The outcome for children with recurrent malignant brain tumors is poor. The majority of patients die of progressive disease within months of relapse, and other therapeutic options are needed. The goal of this Phase I study was to evaluate the safety of in vivo suicide gene therapy in 12 children with recurrent, malignant, supratentorial brain tumors.

Methods. After optimal repeated tumor resection, multiple injections of murine vector--producing cells shedding murine replication--defective retroviral vectors coding the **herpes simplex virus thymidine kinase type 1 (HSV-Tk1)** gene were made into the rim of the resection cavity. Fourteen days after the vector-producing cells were injected, ganciclovir was administered for 14 days. The retroviral vector that was used only integrated and expressed **HSV-Tk1** in proliferating cells, which are killed after a series of metabolic events lead to cell death. The median age of the patients was 11 years (range 2--15 years). Treated brain tumors included seven malignant gliomas, two ependymomas, and three primitive neuroectodermal tumors. The patients were treated with one of three escalating dose concentrations of vector-producer cells. Four transient central nervous system adverse effects were considered possibly related to the vector-producing cells. In no child did permanent neurological worsening or ventricular irritation develop, and tests for replication-competent retroviruses yielded negative findings.

Conclusions. This Phase I study demonstrates that in vivo gene therapy in which a replication-defective retroviral vector in murine vector--producing cells is delivered by brain injections can be performed with satisfactory safety in a select group of children with localized supratentorial brain tumors.

EX VIVO GENE THERAPY FOR SEVERE COMBINED IMMUNODEFICIENCY (SCID)

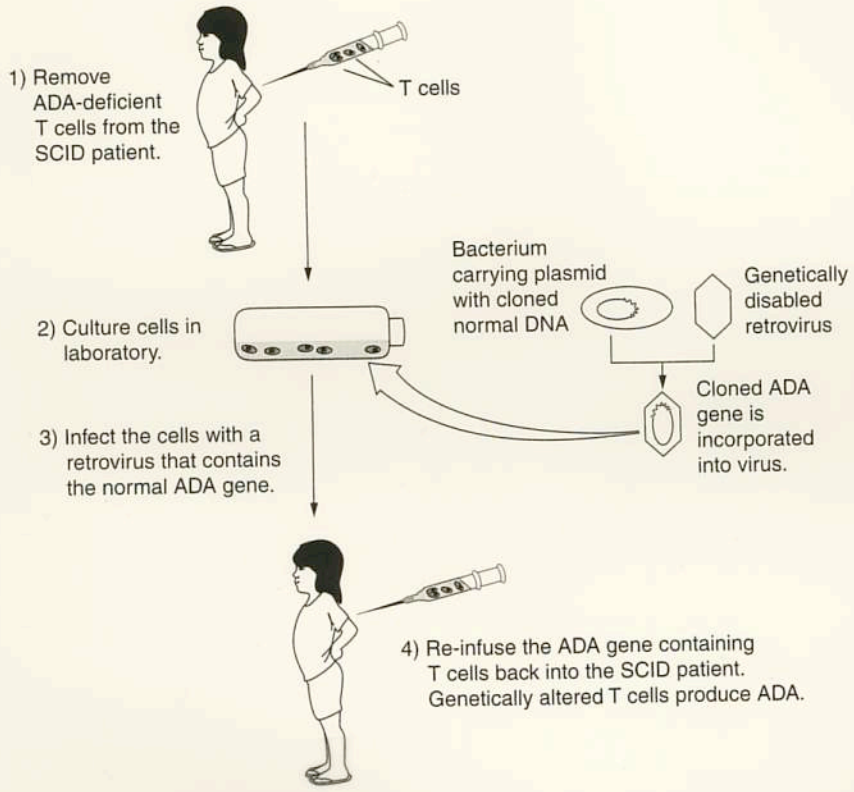


Figure 11.12 The First Human Gene Therapy An *ex vivo* gene therapy strategy was used in a 4-year-old SCID patient with a deficiency in the ADA gene.

55

A RETRO VIRUS

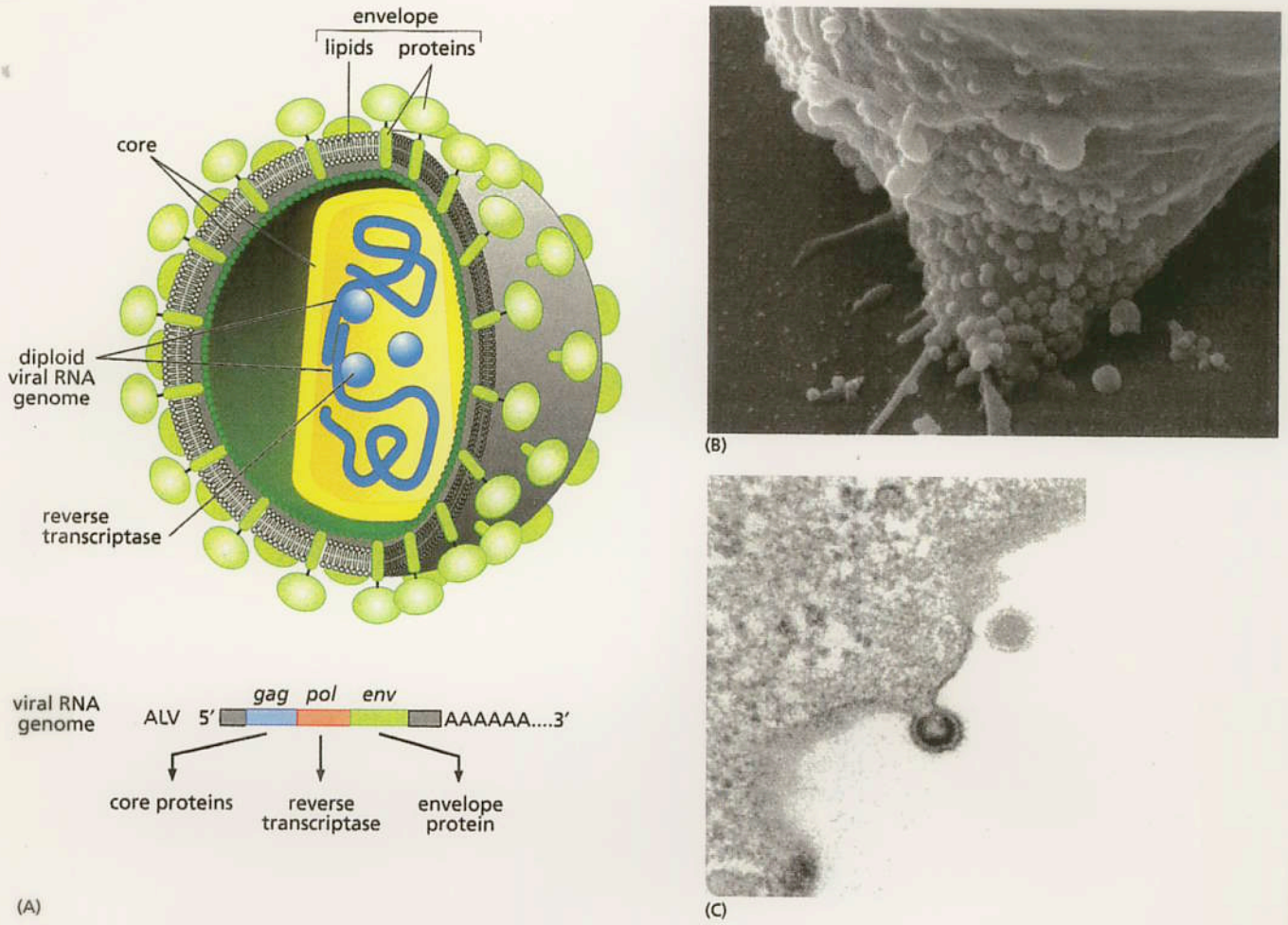
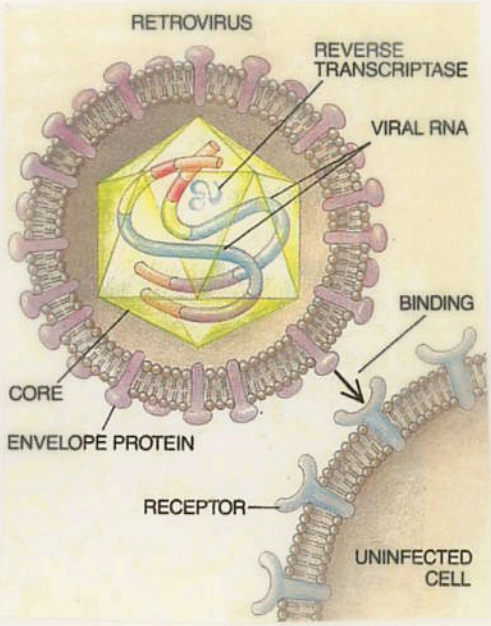
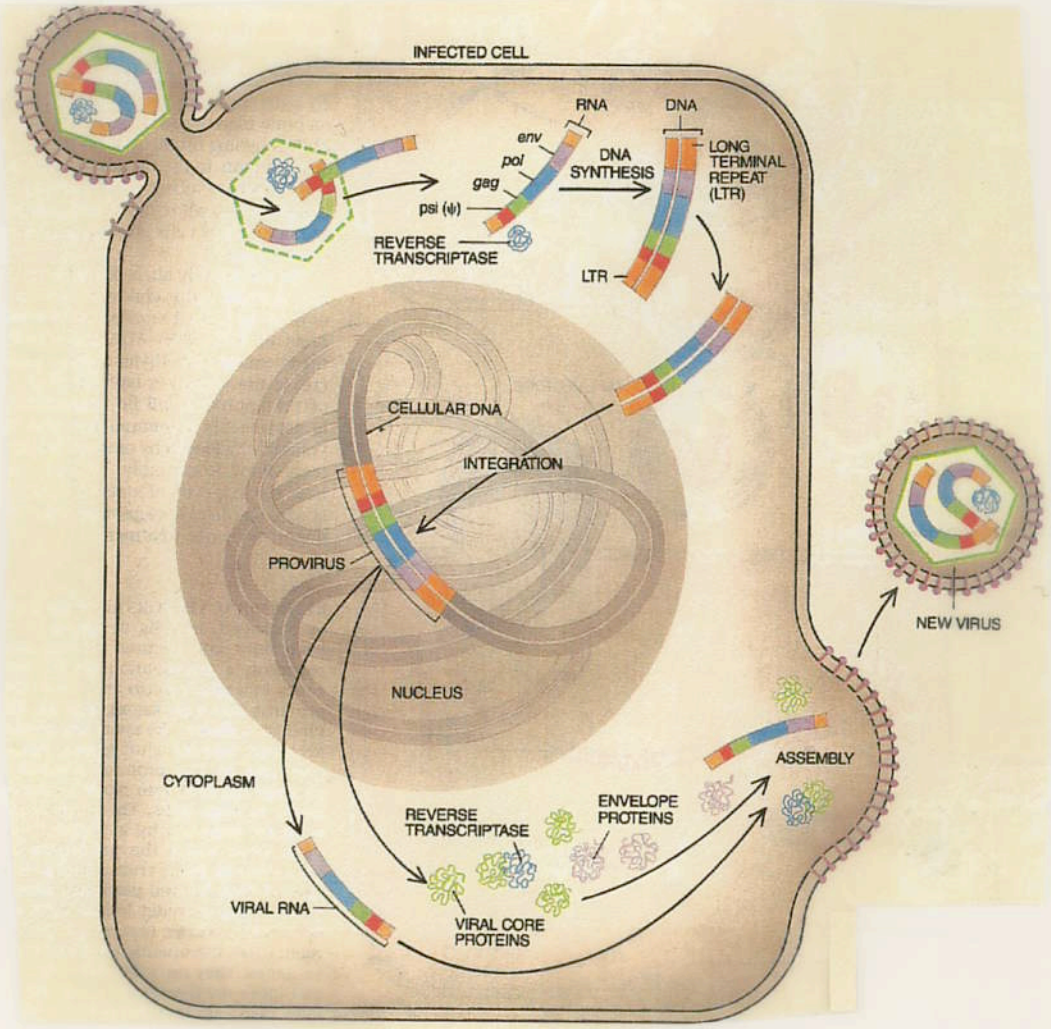


Figure 3.4 The virion of RSV and related viruses (A) This schematic drawing of the structure of a *retrovirus* virion, such as that of Rous sarcoma virus, indicates three major types of viral proteins. The glycoprotein spikes (encoded by the viral *env* gene) protrude from the lipid bilayer that surrounds the virion; these spikes enable the virion to *adsorb* (attach) to the surface of a cell and to introduce the internal contents of the virion into its cytoplasm. These include a complex protein coat formed by the several core proteins encoded by the viral *gag* gene. Within this protein shell are found two identical copies of the viral genomic RNA and a number of reverse transcriptase molecules specified by the viral *pol* gene. (B) Scanning electron micrograph and (C) transmission electron micrograph showing murine leukemia virus (MLV) particles budding from the surface of an infected cell. As the nucleocapsids (containing the gag proteins, the virion RNA, and the reverse transcriptase) leave the cell, they wrap themselves with a patch of lipid bilayer taken from the plasma membrane of the infected cell. (A, adapted from H. Fan et al., The Biology of AIDS. Boston, MA: Jones and Bartlett Publishers, 1989; B, courtesy of Albert Einstein College of Medicine; C, courtesy of Laboratoire de Biologie Moleculaire.)

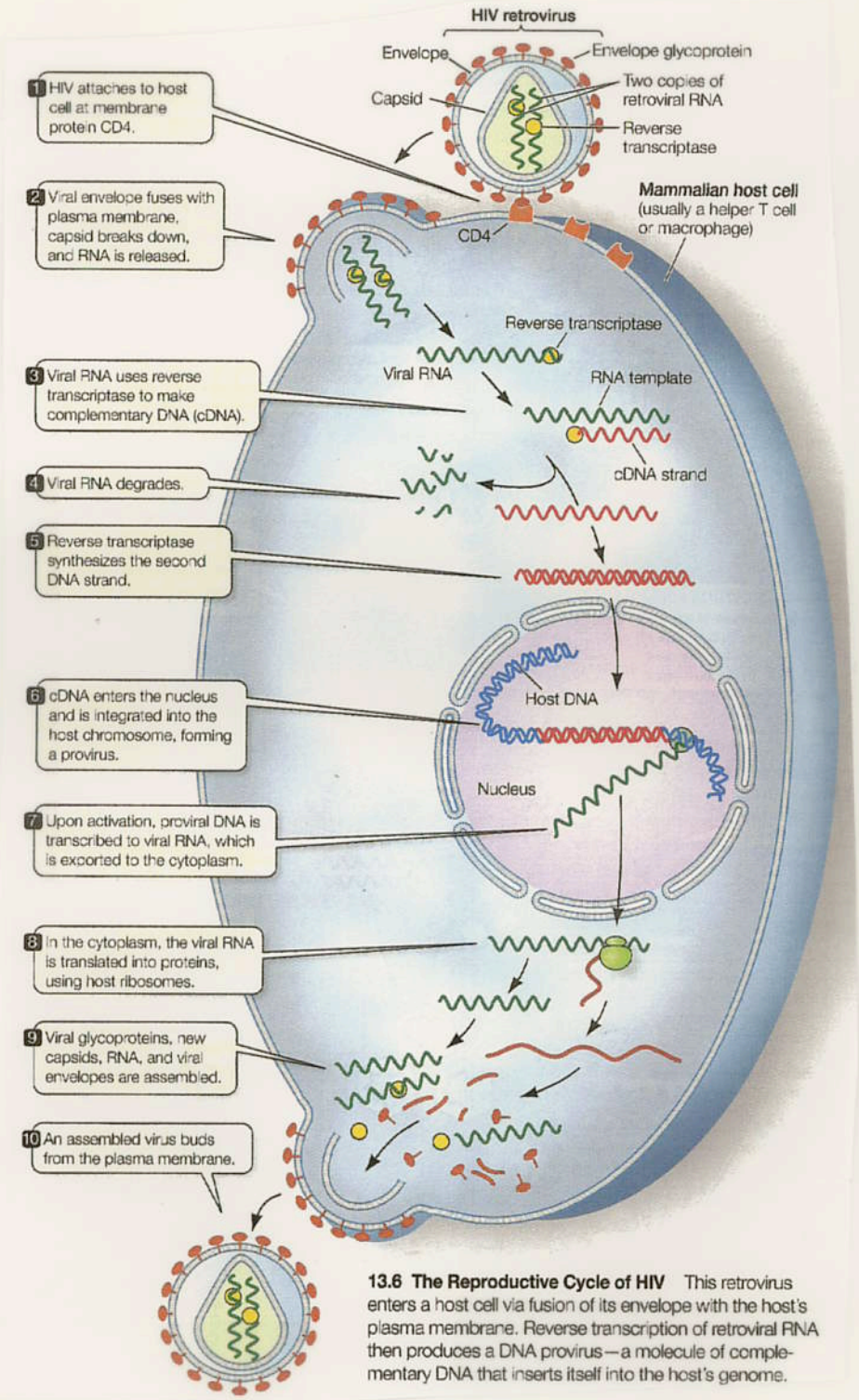
Figure 3.2 Rous's protocol for inducing sarcomas in chickens Rous removed a sarcoma from the breast muscle of a chicken, ground it with sand, and passed the resulting homogenate through a fine-pore filter. He then injected the filtrate (the liquid that passed through the filter) into the wing web of a young chicken and observed the development of a sarcoma many weeks later. He then ground up this new sarcoma and repeated the cycle of homogenization, filtration, and injection, once again observing a tumor in another young chicken. These cycles could be repeated indefinitely; after repeated serial passaging, the virus was able to produce sarcomas far more rapidly than the original viral isolate.

RETROVIRUS LIFE CYCLE



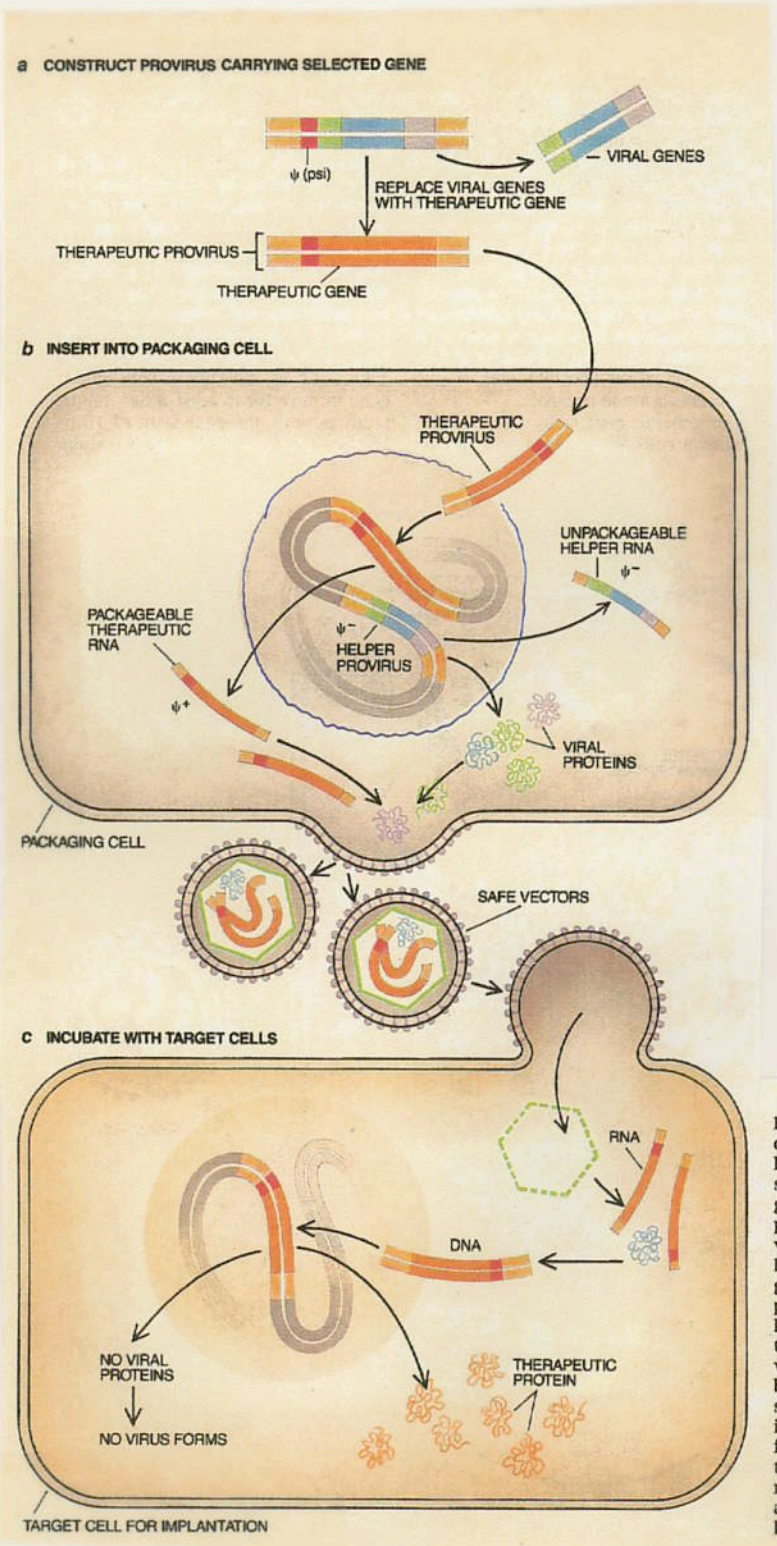


HIV IS A RETROVIRUS



13.6 The Reproductive Cycle of HIV This retrovirus enters a host cell via fusion of its envelope with the host's plasma membrane. Reverse transcription of retroviral RNA then produces a DNA provirus—a molecule of complementary DNA that inserts itself into the host's genome.

USING RETROVIRUSES FOR EX VIVO GENE THERAPY



RETROVIRAL VECTORS are assembled, or packaged, in cells designed to release only safe vectors. Investigators substitute a therapeutic gene for viral genes in a provirus (*a*) and insert that provirus into a packaging cell (*b*). The viral DNA directs the synthesis of viral RNA but, lacking viral genes, cannot give rise to the proteins needed to package the RNA into particles for delivery to other cells. The missing proteins are supplied by a "helper" provirus from which the psi region has been deleted. Psi is crucial to the inclusion of RNA in viral particles; without it, no virus carrying helper RNA can form. The particles that escape the cell, then, carry therapeutic RNA and no viral genes. They can enter other cells (*c*) and splice the therapeutic gene into cellular DNA, but they cannot reproduce.

Types of Human Gene Therapy Clinical Trials

TABLE 12.4 GENE THERAPIES BEING STUDIED IN CANCER PATIENTS THAT MAY RECEIVE PATENTS AND REGULATORY APPROVAL

Approach	*Number of U.S. Trials* Approved since 1988 or Awaiting Federal Approval
Antisense therapy (to block synthesis of proteins encoded by deleterious genes)	4
Chemoprotection (to add proteins to normal cells to protect them from chemotherapies)	7
Immunotherapy (to enhance the body's immune defenses against cancer)	58
Pro-drug, or suicide gene therapy (to render cancer cells highly sensitive to selected drugs)	21
Tumor suppressor genes (to replace a lost or damaged cancer-blocking gene)	6
Antibody genes (to interfere with the activity of cancer-related proteins in tumor cells)	2
Oncogene down-regulation (to shut off genes that favor uncontrolled growth and spread of tumor cells)	2

Source: Fiattman, G. I., and Kaplan, J. M. (2001). "Patenting Expressed Sequence Tags and Single Nucleotide Polymorphisms," *Nature Biotechnology* 19: 683.

APPROVED GENE THERAPY TRIALS

Table 1. Conditions in which human gene transfer has been approved

Monogenic disorders	**Cancer**
Cystic fibrosis	Gynaecological: breast, ovary, cervix
SCID	Nervous system: glioblastoma, leptomeningeal
Haemophilia A and B	carcinomatosis, glioma, astrocytoma, neuroblastoma
Hurler syndrome	Gastro-intestinal: colon, colorectal, liver
Hunter syndrome	metastases, post-hepatitis liver cancer
Huntington's chorea	Genito urinary: prostate, renal
Duchenne Muscular Dystrophy	Skin: melanoma
Canavan disease	Head and neck
Chronic granulomatous disease	Lung: adenocarcinoma, small cell, non small cell
Familial hypercholesterolaemia	Mesothelioma
Gaucher disease	Haematological: leukaemia, lymphoma, multiple
Fanconi's anaemia	Myeloma
Purine nucleoside phosphorylase deficiency	Sarcoma
Ornithine transcarbamylase deficiency	Germ cell tumors
Leukocyte adherence deficiency	
Gyrate atrophy	
Fabry disease	
Amyotrophic lateral sclerosis	**Other diseases**
Junctional epidermolysis bullosa	Inflammatory bowel disease
	Rheumatoid arthritis
Vascular disease	Chronic renal disease
Peripheral arterial disease	Carpal tunnel syndrome
Coronary heart disease	Alzheimer's disease
Venous ulcers	Fractures
Vascular complications of diabetes	Diabetic neuropathy
	Parkinson's disease
Infectious disease	Erectile disfunction
HIV/AIDS	Superficial corneal opacity
Tetanus	Retinitis pigmentosa
CMV infection	Glaucoma
Adenovirus infection	

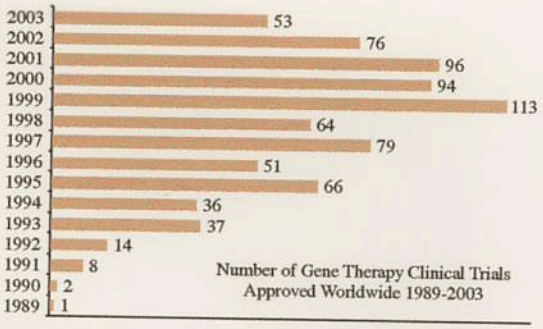


Figure 1. New trials approved by year 1989–2003

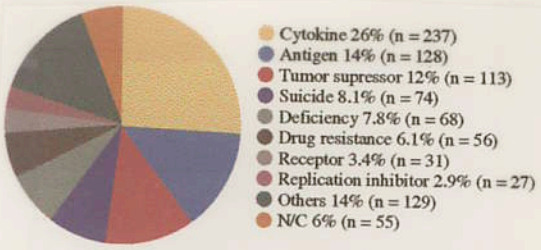
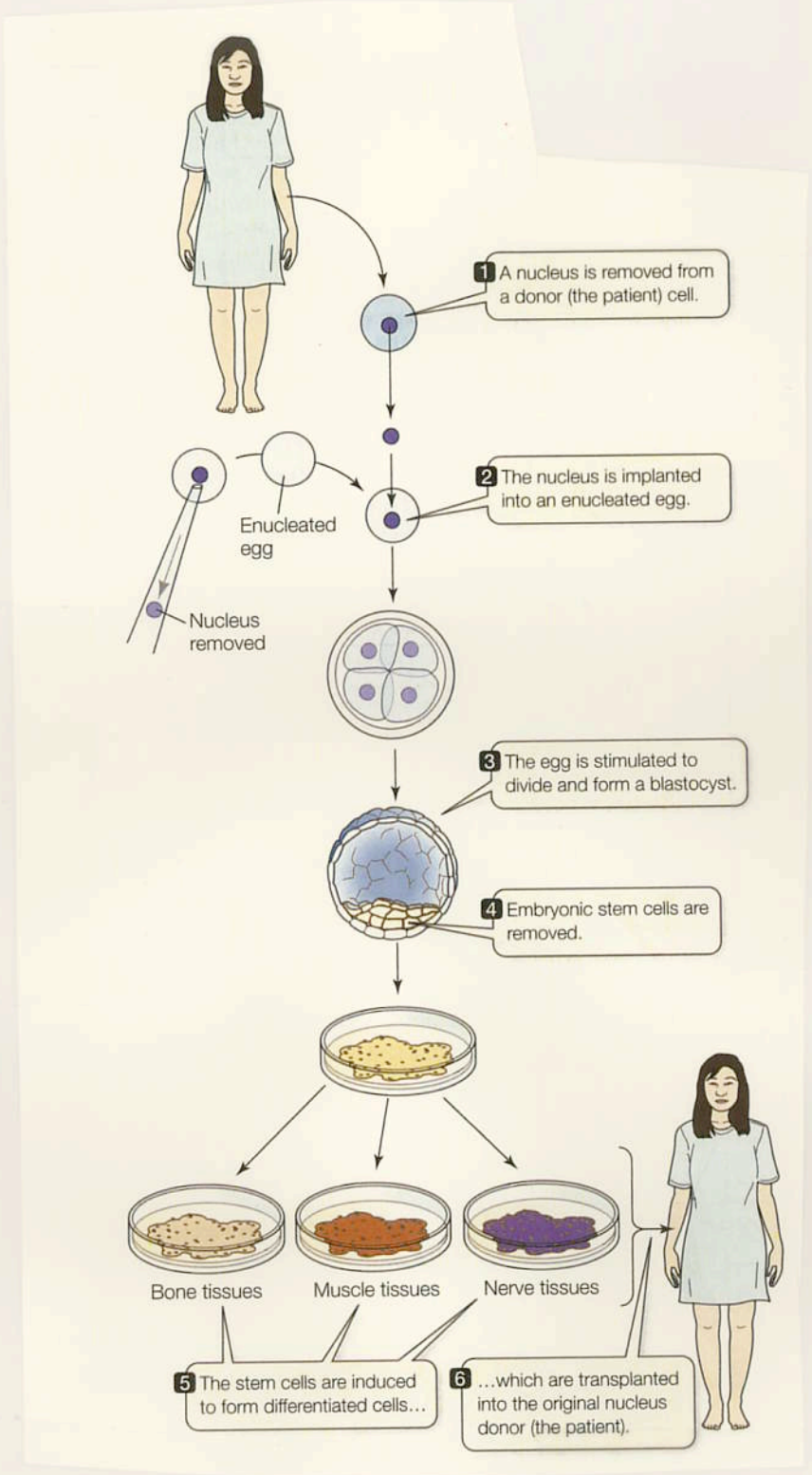


Figure 5. Distribution of gene therapy clinical trials by gene. N/C = not communicated

J Gene Med 2004; 6: 597–602.

COMBINING GENE Therapy with STEM CELLS & Therapeutic Cloning



Genetic Engineer Cells Before Nuclear or Cell Transfer

19.8 Therapeutic Cloning The combination of nuclear transplantation and stem cell technologies could lead to the production of cells and tissues for transplantation that would not be rejected by the patient's immune system.

Human Gene Therapy Issues

- Regulation
- Consent
- Risks
- Enhancement
- Eugenics